beagle_quenching_test_20180607

Stephen Shivers, Betty, Kreakie, & Bryan Milstead

12 June 2018

Introduction

There is concern that the beagle fluorometers are not reading chlorophyll and phycocyanin values correctly at high concentrations. This may be caused by physical interference (quenching) at high concentrations. If this is the case measured concentrations should increase if the samples are diluted. To test this we conducted a series of measurements on a lab culture of *Microcystis*. To look at quenching we compared values for undiluted samples to those from a dilution series. We were also interested in testing whether dark adaptation and freezing affected the results.

Methods

- A single laboratory culture of *Microcystis* was used for all tests. On the first day of the study we measured concentrations of microcystin and chlorophyll with the beagles on the fresh, unprocessed samples following the CMC standard procedure. Triplicate measurements were made on each of two beagles. To check for quenching samples were measured undiluted, and 10x (1:9), 100x (1:99) and 1000x (1:999) dilutions.
- Triplicate 10 ml samples were placed in the dark overnight to check for dark adaption. Phycocyanin and chlorophyll were measured for these samples the following day. As with the fresh samples the dark adapted samples were measured undiluted, and 10x (1:9), 100x (1:99) and 1000x (1:999) dilutions on two beagles.
- Six 10 ml samples (2 beagles x 3 reps) were frozen overnight and then measured the following day following a stepped 50% dilution series that ranged from 2x - 1024x dilutions (see below).
- Fresh samples were also processed for chlorophyll and phycocyanin extaction with measurement of concentration using a Turner benchtop fluorometer.
- *Microcystis* cells counts were done with a hemocytometer at 100x magnification. A total of 3 4x4 grids were counted by two observers (Stephen and Bryan).

steps

1. Prep for extracted chla and pc (10 ml x 2 extractions x 3 reps): for each culture place 10 ml in each of three scintillation vials and store in darkness overnight. Sum = 60 ml.
2. Unfrozen, non-dark adapted samples of each culture (10 ml x 3 reps x 2 beagles). Sum = 60 ml. For each rep:
   - Read RFU, Chla, and PC (4 + 4 ml) on beagles
   - Dilute 1:9 (1 ml undiluted sample + 9 ml di water) and Read RFU, Chla, and PC
   - Dilute 1:99 (1 ml 1:9 dilution + 9 ml di water) and Read RFU, Chla, and PC
   - Dilute 1:999 (1 ml 1:99 dilution + 9 ml di water) and Read RFU, Chla, and PC
3. Unfrozen, dark adapted samples of each culture (10 ml x 3 reps x 2 beagles): for each culture place 20 ml in each of three scintillation vials and store in darkness overnight. Sum = 60 ml. For each rep:
   - Read RFU, Chla, and PC (4 + 4 ml) on beagles
   - Dilute 1:9 (1 ml undiluted sample + 9 ml di water) and Read RFU, Chla, and PC
   - Dilute 1:99 (1 ml 1:9 dilution + 9 ml di water) and Read RFU, Chla, and PC
   - Dilute 1:999 (1 ml 1:99 dilution + 9 ml di water) and Read RFU, Chla, and PC

4. Frozen samples (10 ml x 3 reps x 2 beagles): For each culture place 10 ml in each of six scintillation vials and freeze overnight. Sum = 60 ml. Thaw each vial and:
    - Read RFU, Chla, and PC (4 ml) on beagles
    - Dilute 1:1 (5 ml undiluted sample + 5 ml di water) and Read RFU, Chla, and PC
    - Dilute 1:3 (5 ml previous dilution + 5 ml di water) and Read RFU, Chla, and PC
    - Dilute 1:7 (5 ml previous dilution + 5 ml di water) and Read RFU, Chla, and PC
    - Dilute 1:15 (5 ml previous dilution + 5 ml di water) and Read RFU, Chla, and PC
    - Dilute 1:31 (5 ml previous dilution + 5 ml di water) and Read RFU, Chla, and PC
    - Dilute 1:63 (5 ml previous dilution + 5 ml di water) and Read RFU, Chla, and PC
    - Dilute 1:127 (5 ml previous dilution + 5 ml di water) and Read RFU, Chla, and PC
    - Dilute 1:255 (5 ml previous dilution + 5 ml di water) and Read RFU, Chla, and PC
    - Dilute 1:511 (5 ml previous dilution + 5 ml di water) and Read RFU, Chla, and PC
    - Dilute 1:1023 (5 ml previous dilution + 5 ml di water) and Read RFU, Chla, and PC

Materials

- Laboratory culture of *Microcystis*
- Two beagles
- Sample: 240 ml of each sample
- 1, 5, and 10 ml pipettors and tips
- scintillation vials
- cuvettes
- scintillation vials
- di water for dilutions

Results

Cells Counts

- The same samples were counted by Stephen and Bryan with different results. Bryan's eyesight is not what it used to be so perhaps better to go with Stephen's counts.
- To convert these to cells / ml multiply by 10k.
- The cell count is between 6-7 million cells / ml
- Fairly dense culture

who	n	mean	sd	min	max
bryan	3	628	9.643651	617	635
stephen	3	725	7.937254	716	731

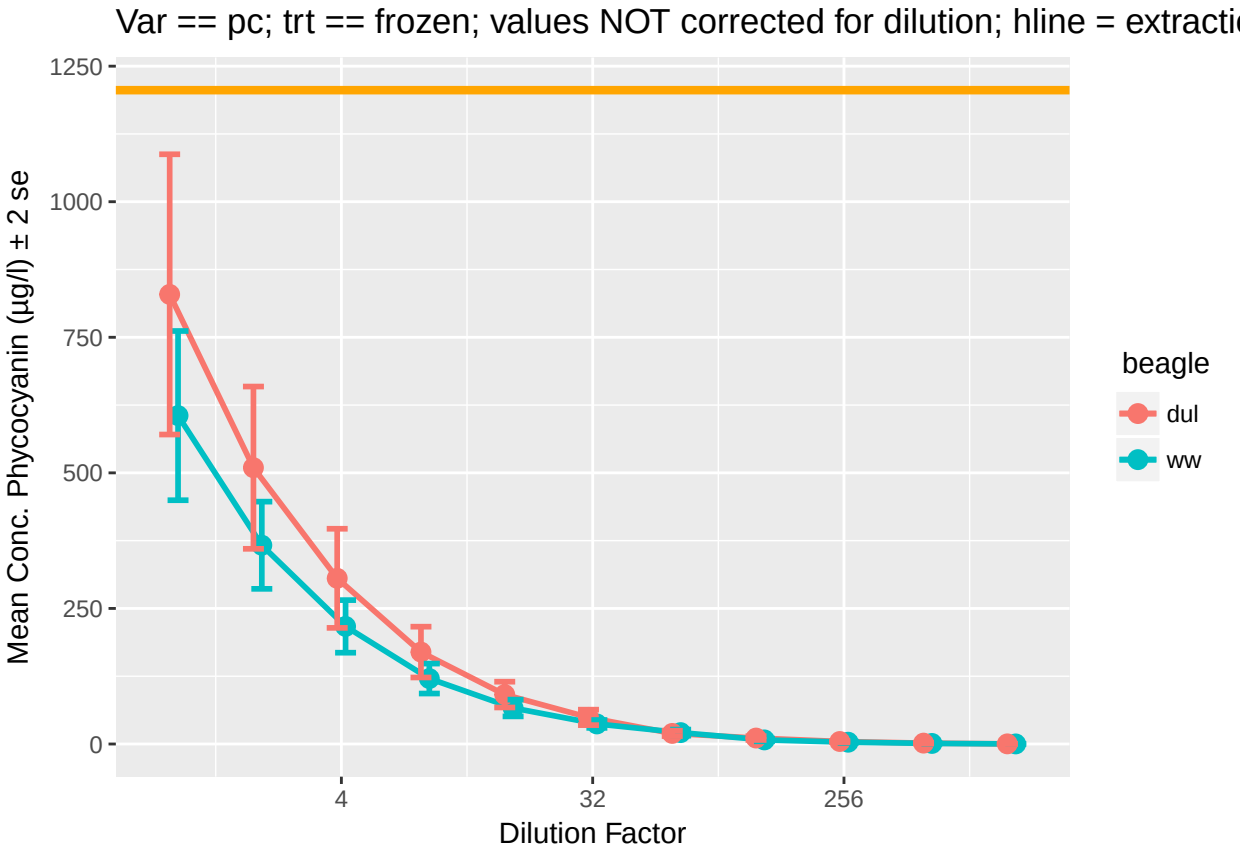
Extractions

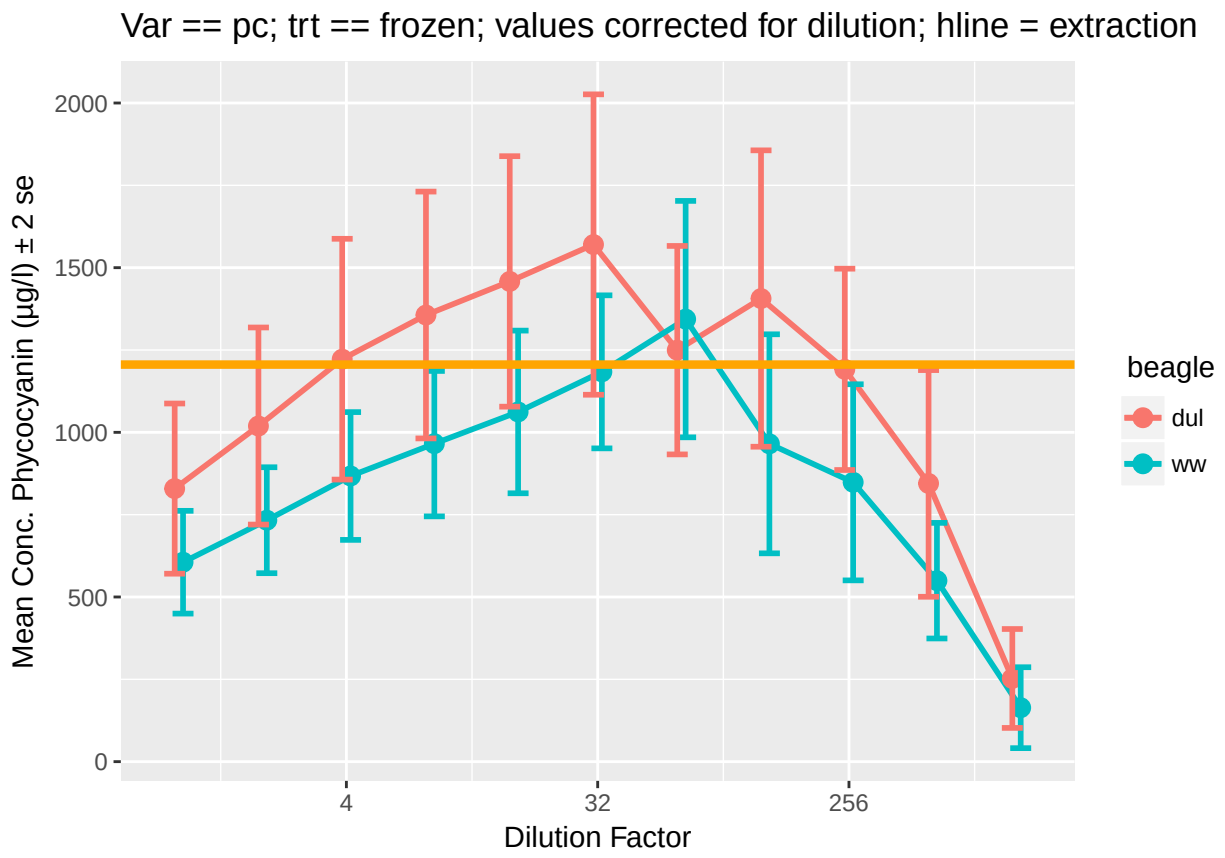
- A summary of the extraction results is presented below
- Values for both are consistent and high

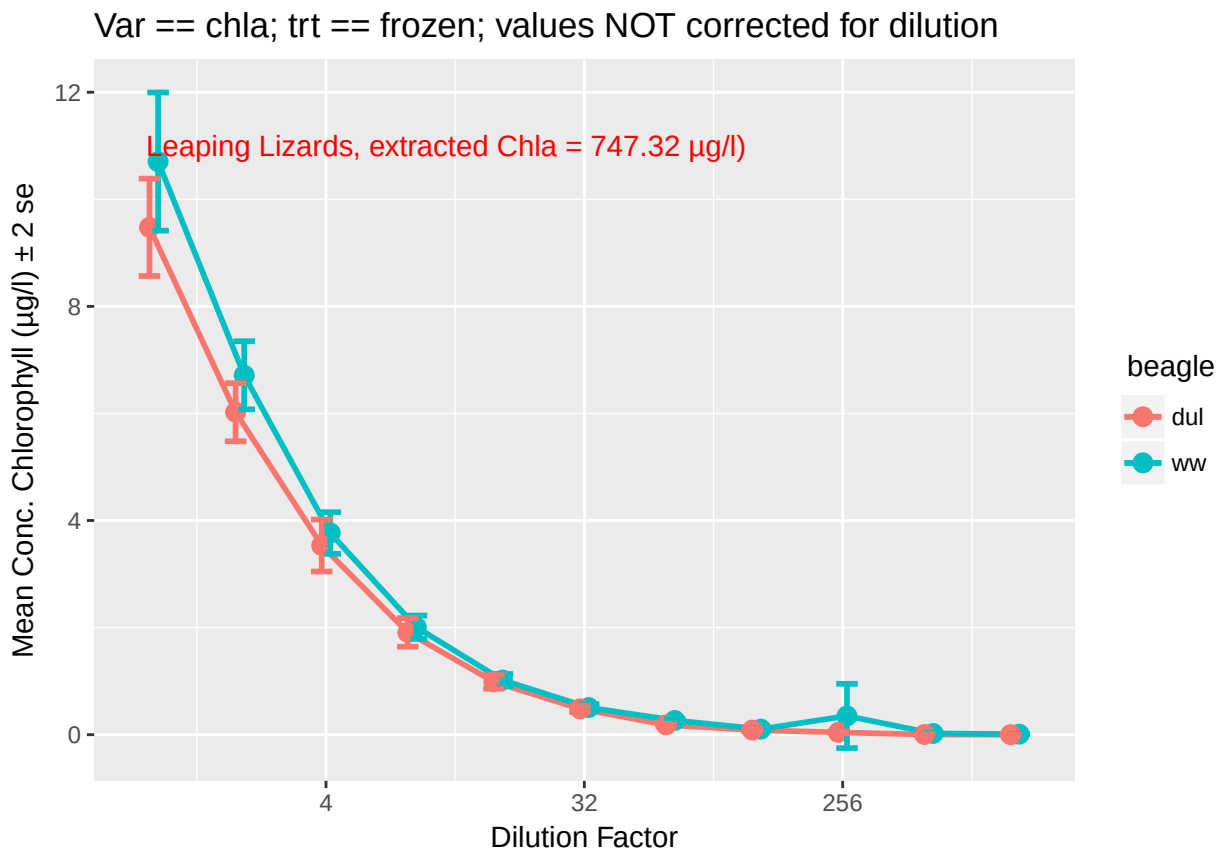
variable	n	mean	sd	min	max
chlorophyll	3	747.320	64.77213	698.83	820.88
phycocyanin	3	1205.713	53.73106	1173.91	1267.75

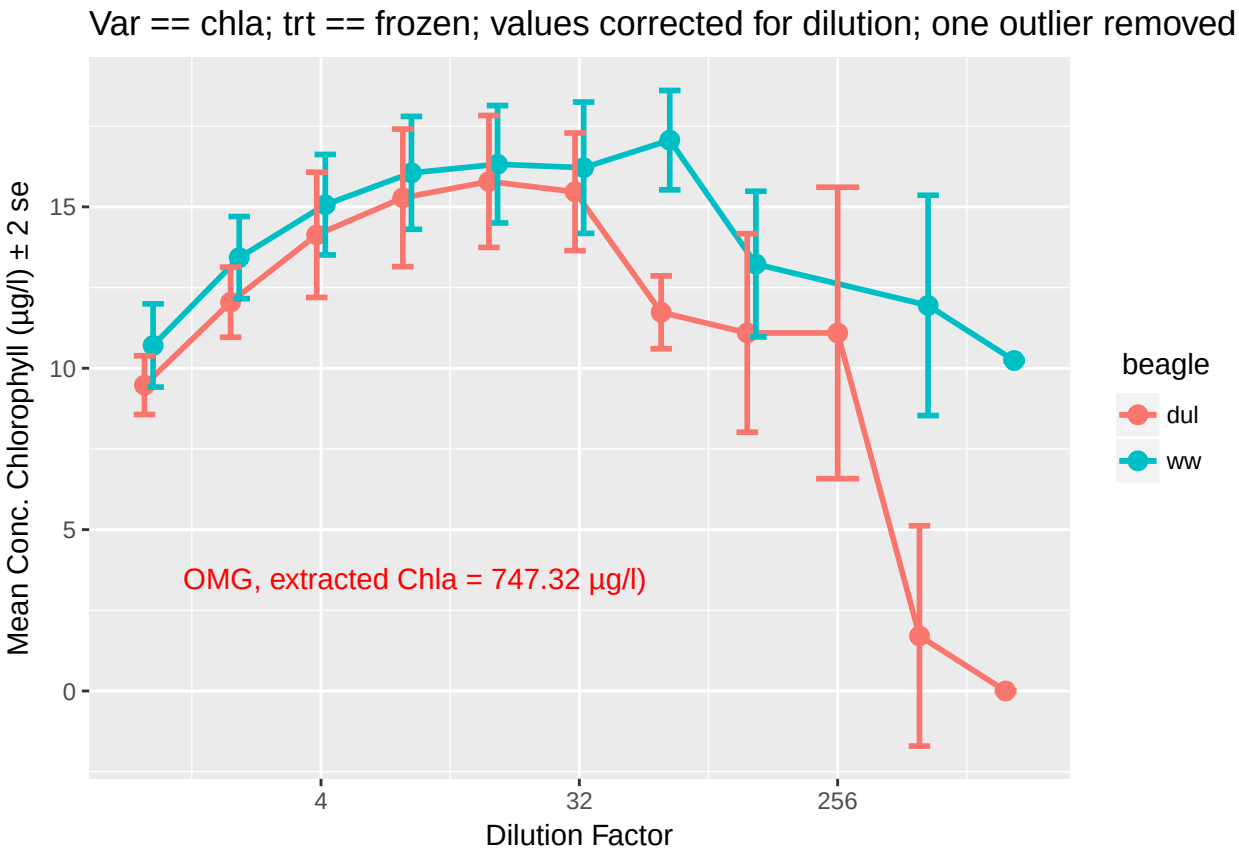
quenching

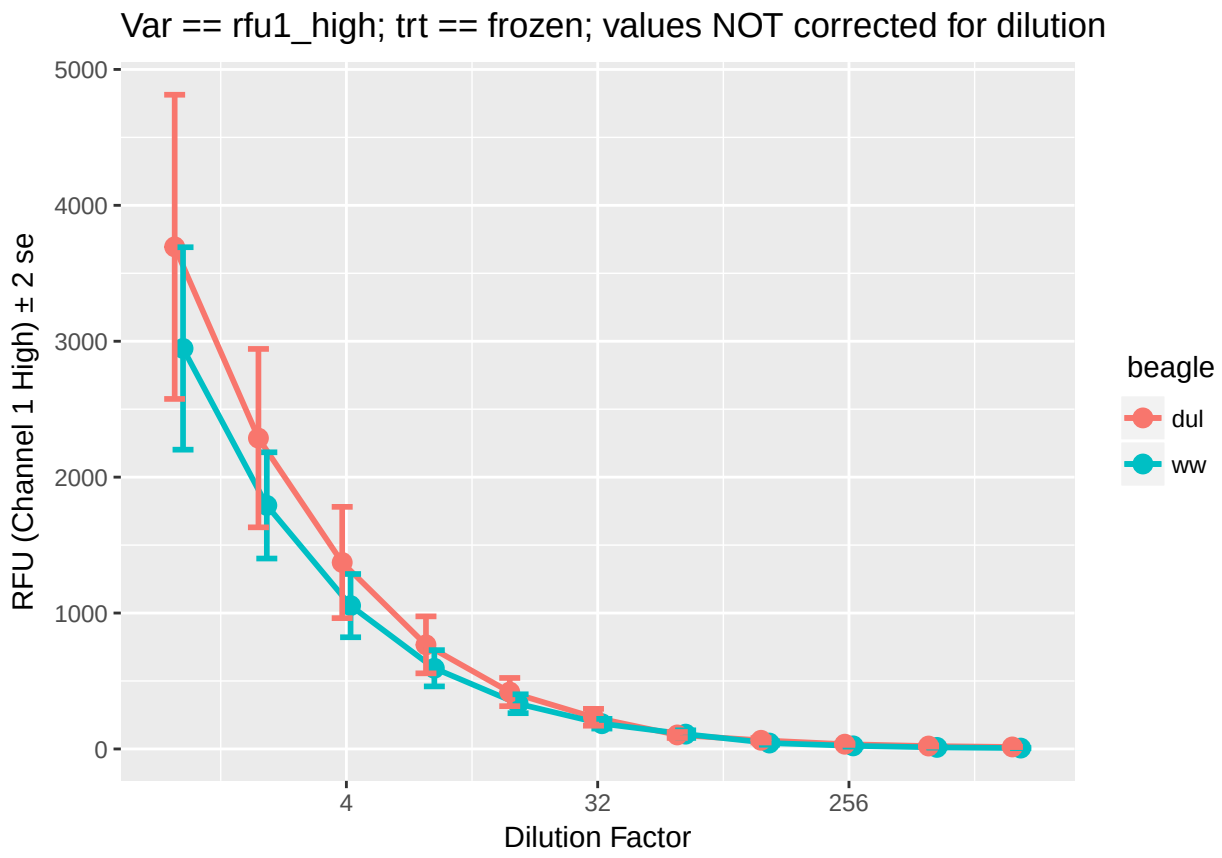
- Since we have a high concentration of Phycocyanin and Chlorophyll a as well as high cell counts we should expect that undiluted samples will show quenching or interference so the the values should increase with dilution.
- The data are plotted two ways.
  - First we plot the raw data against the dilution factor (log2 scale)
  - Then the raw data are corrected for dilution by multiplying by the dilution factor and reploted.
  - Presented are the data for the frozen samples for all six Beagle outputs (Chla, PC, RFU1_high, RFU1_lo, RFU2_high, and RFU2_lo)



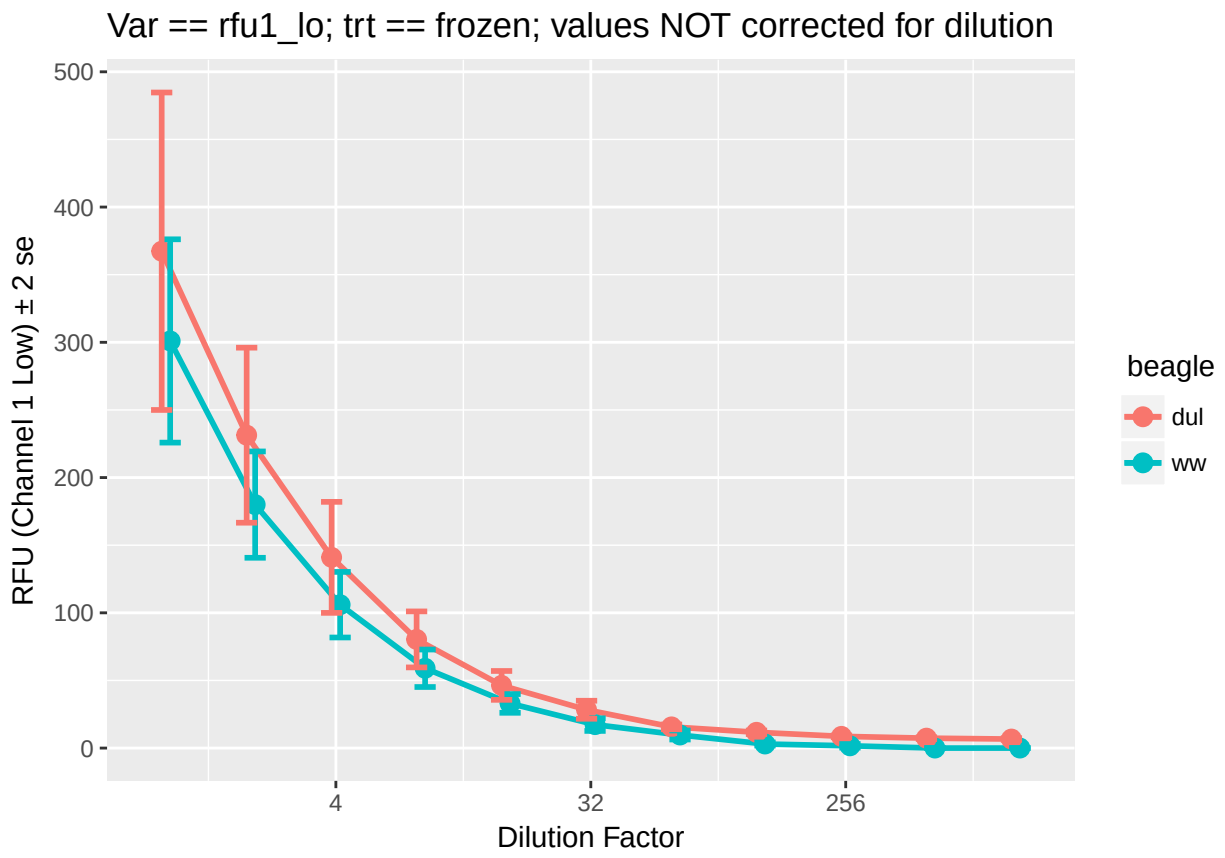

Var == pc; trt == frozen; values corrected for dilution; hline = extraction
Mean Conc. Phycocyanin (µg/l) ± 2 se
2000
1500
1000
500
0
4
32
256
Dilution Factor
beagle
dul
ww

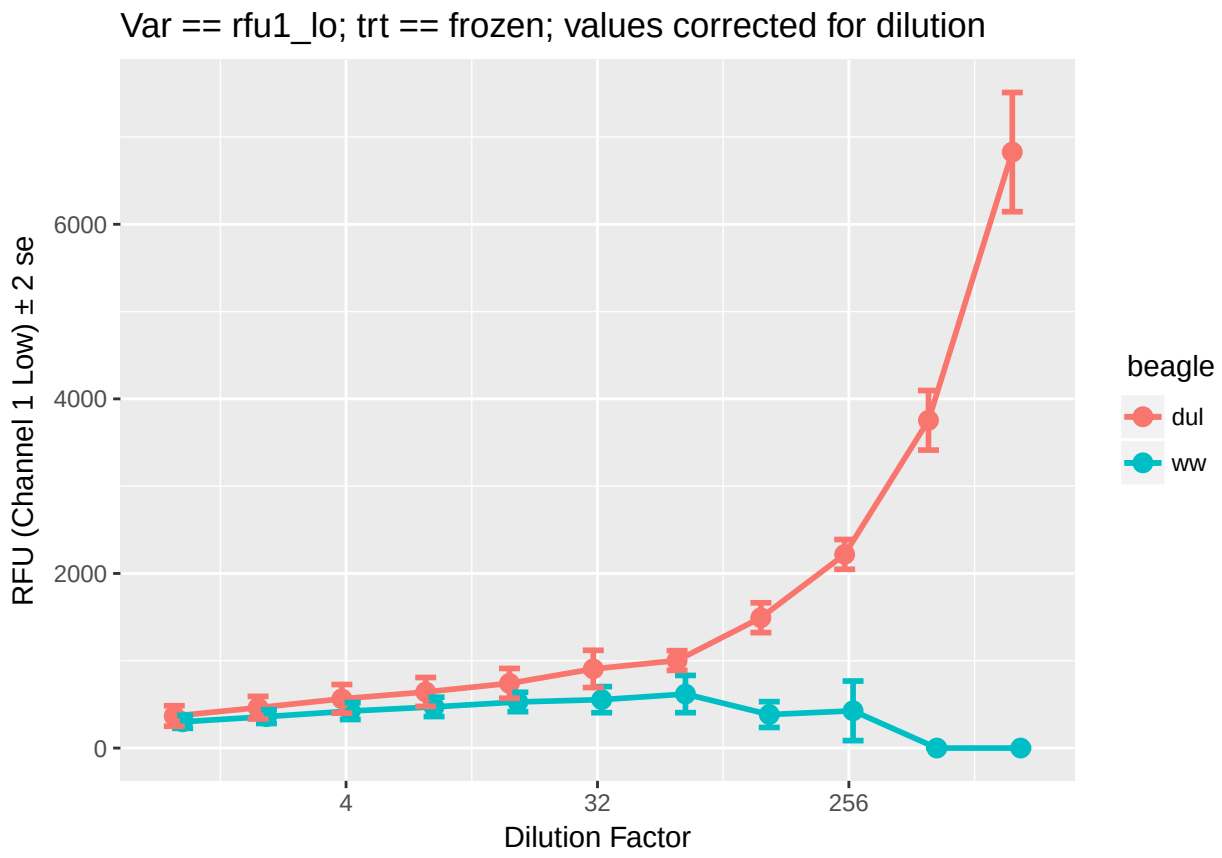

Var == chla; trt == frozen; values NOT corrected for dilution
Leaping Lizards, extracted Chla = 747.32 µg/l)
Mean Conc. Chlorophyll (µg/l) ± 2 se
12
8
4
0
4
32
256
Dilution Factor
beagle
dul
ww

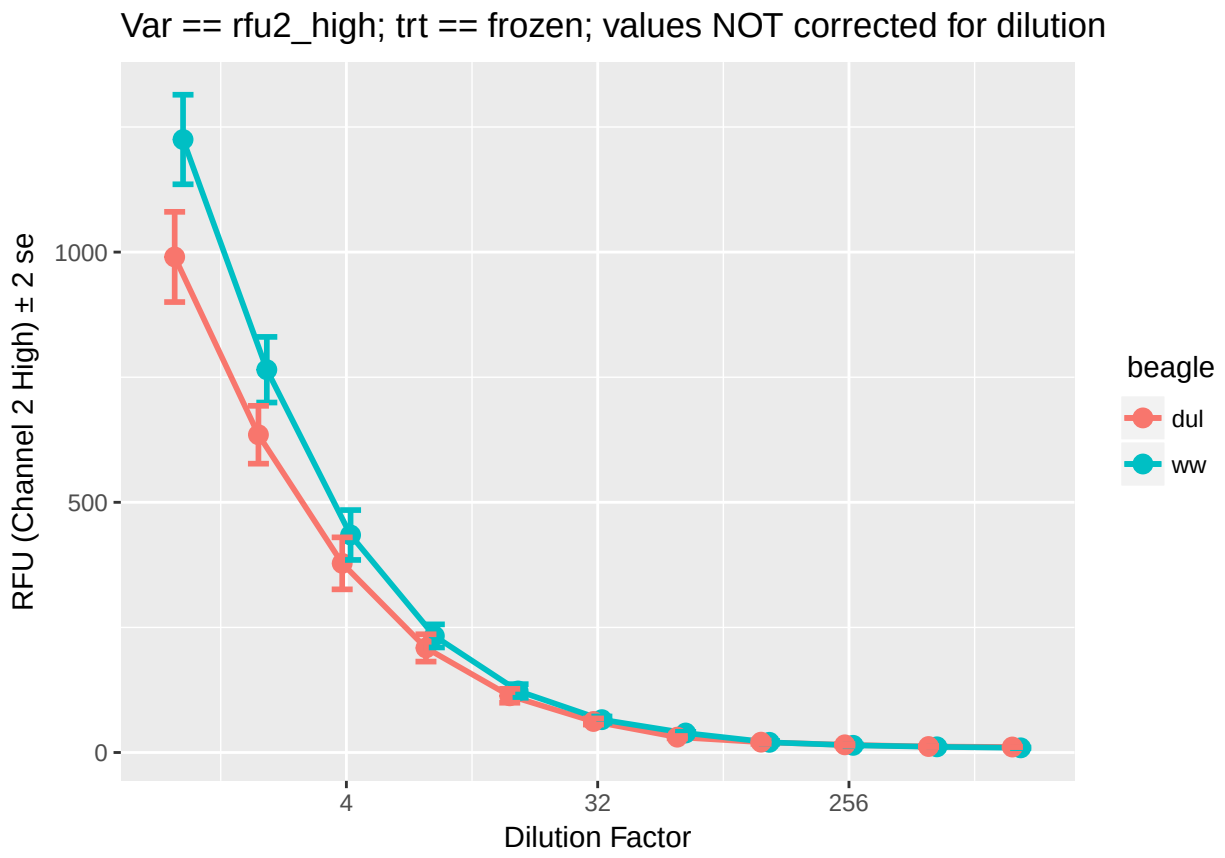

Var == chla; trt == frozen; values corrected for dilution; one outlier removed
Mean Conc. Chlorophyll (µg/l) ± 2 se
15
10
5
0
OMG, extracted Chla = 747.32 µg/l)
4
32
256
Dilution Factor
beagle
dul
ww

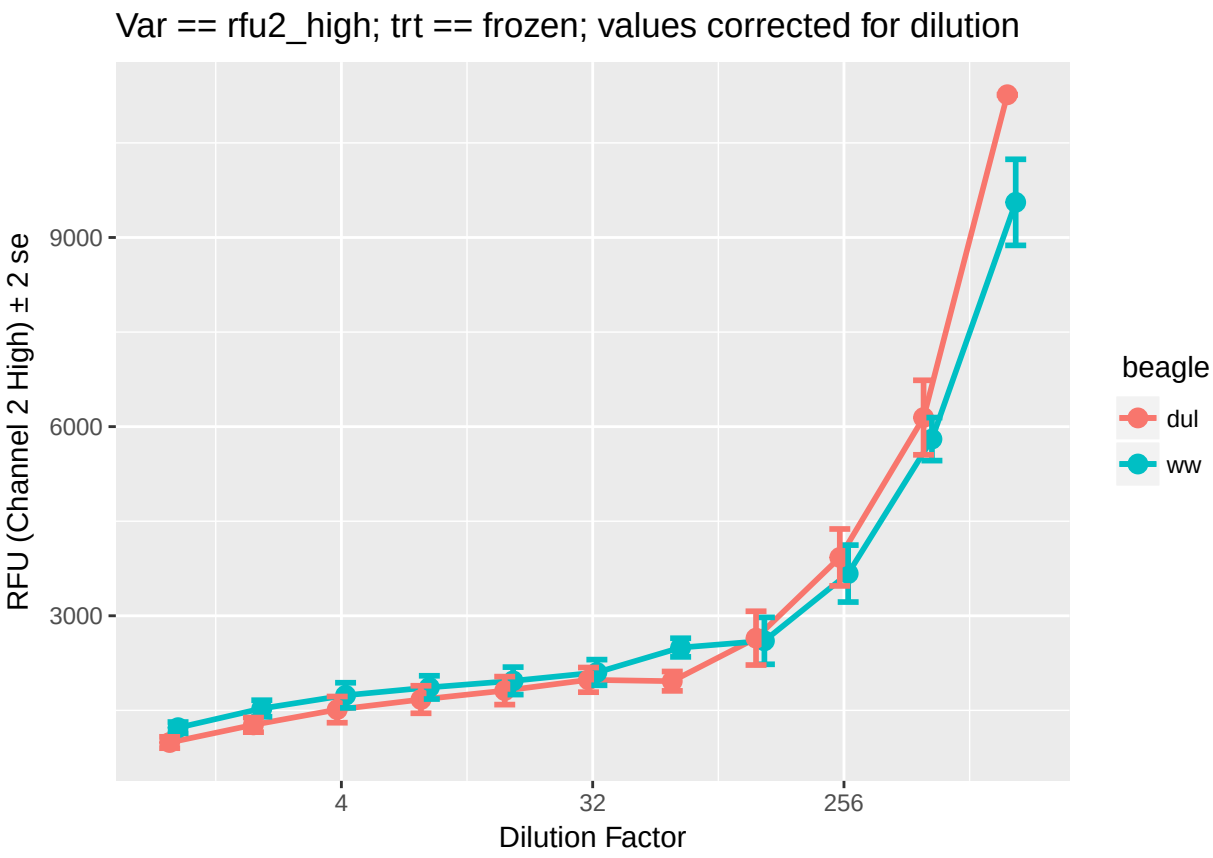

Var == rfu1_high; trt == frozen; values NOT corrected for dilution
RFU (Channel 1 High) ± 2 se
5000
4000
3000
2000
1000
0
4
32
256
Dilution Factor
beagle
dul
ww

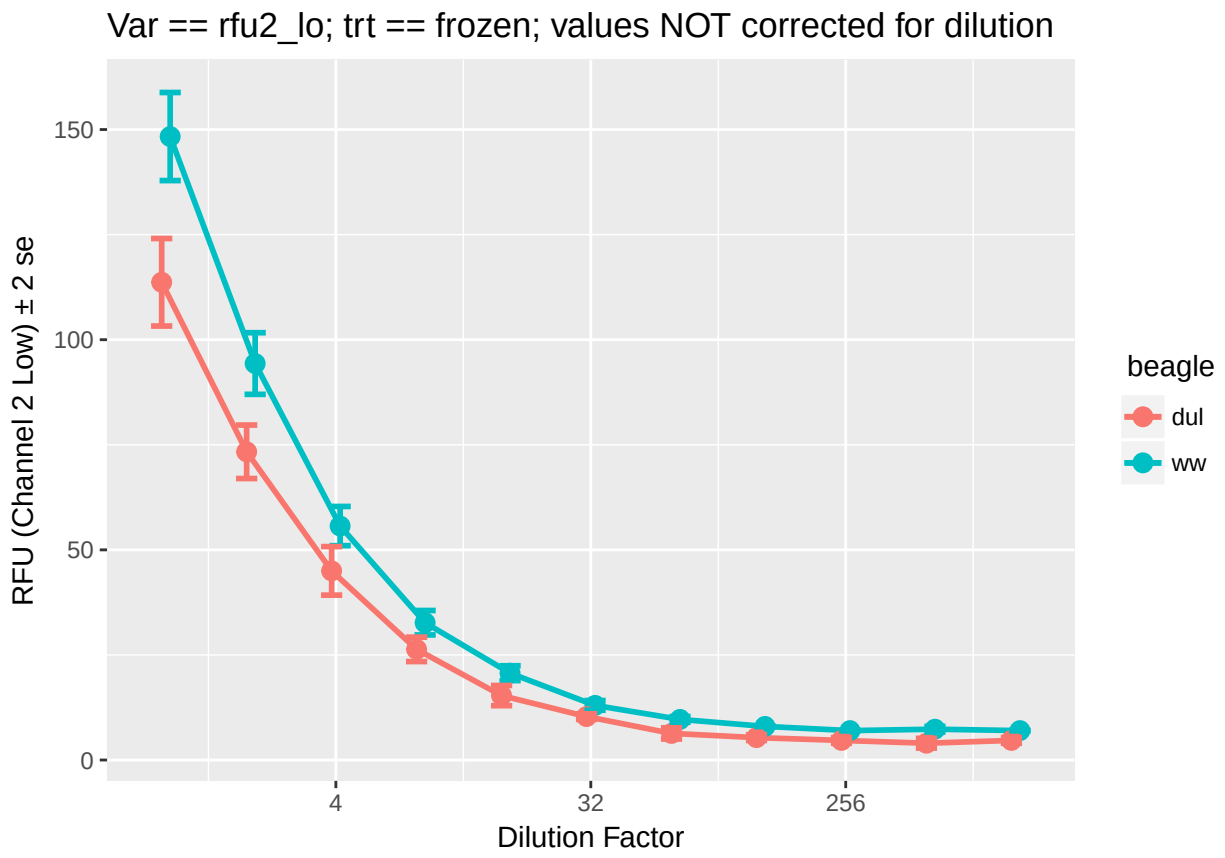

Var == rfu1_high; trt == frozen; values corrected for dilution
RFU (Channel 1 High) ± 2 se
16000
12000
8000
4000
4
32
256
Dilution Factor
beagle
dul
ww

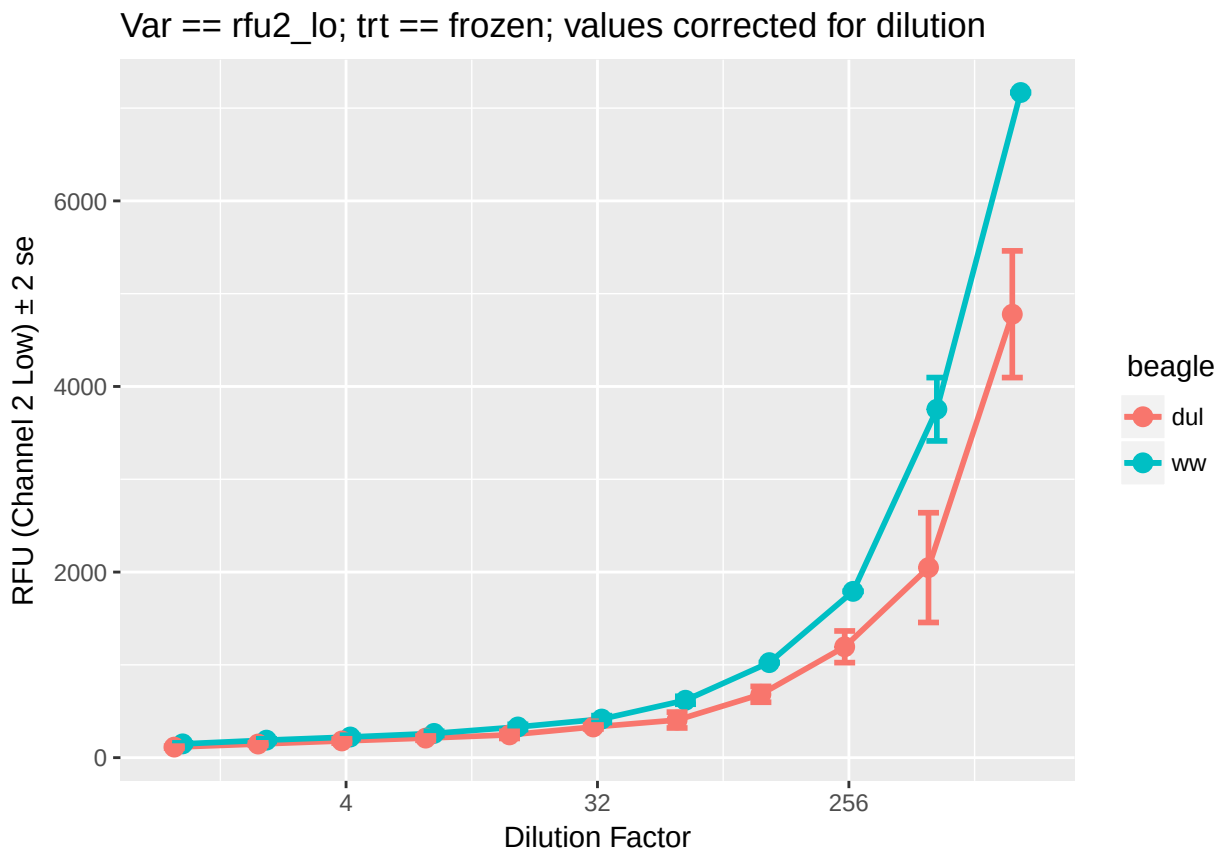

Var == rfu1_lo; trt == frozen; values NOT corrected for dilution
RFU (Channel 1 Low) ± 2 se
500
400
300
200
100
0
4
32
256
Dilution Factor
beagle
dul
ww


Var == rfu1_lo; trt == frozen; values corrected for dilution
RFU (Channel 1 Low) ± 2 se
6000
4000
2000
0
4
32
256
Dilution Factor
beagle
dul
ww


Var == rfu2_high; trt == frozen; values NOT corrected for dilution
RFU (Channel 2 High) ± 2 se
1000
500
0
4
32
256
Dilution Factor
beagle
dul
ww

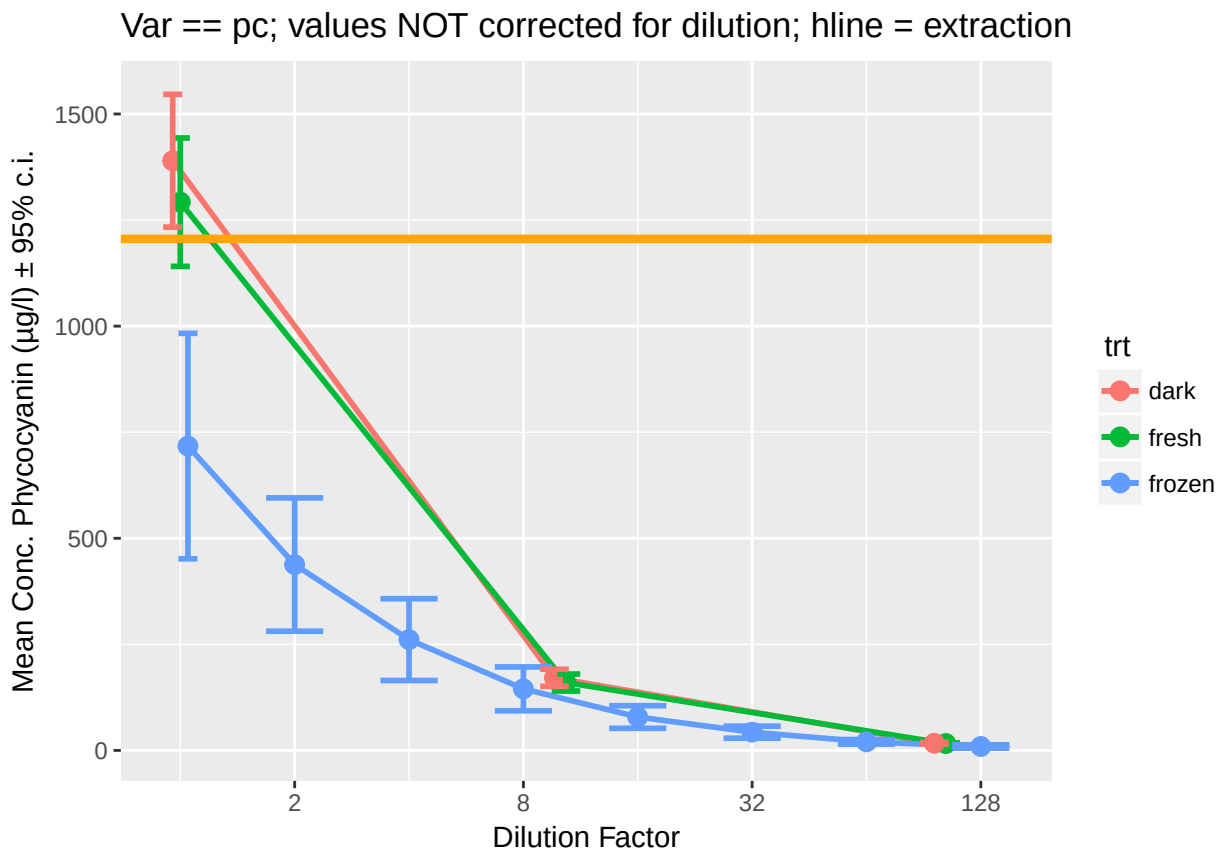

Var == rfu2_high; trt == frozen; values corrected for dilution
RFU (Channel 2 High) ± 2 se
9000
6000
3000
4
32
256
Dilution Factor
beagle
dul
ww


Var == rfu2_lo; trt == frozen; values NOT corrected for dilution
RFU (Channel 2 Low) ± 2 se
150
100
50
0
4
32
256
Dilution Factor
beagle
dul
ww

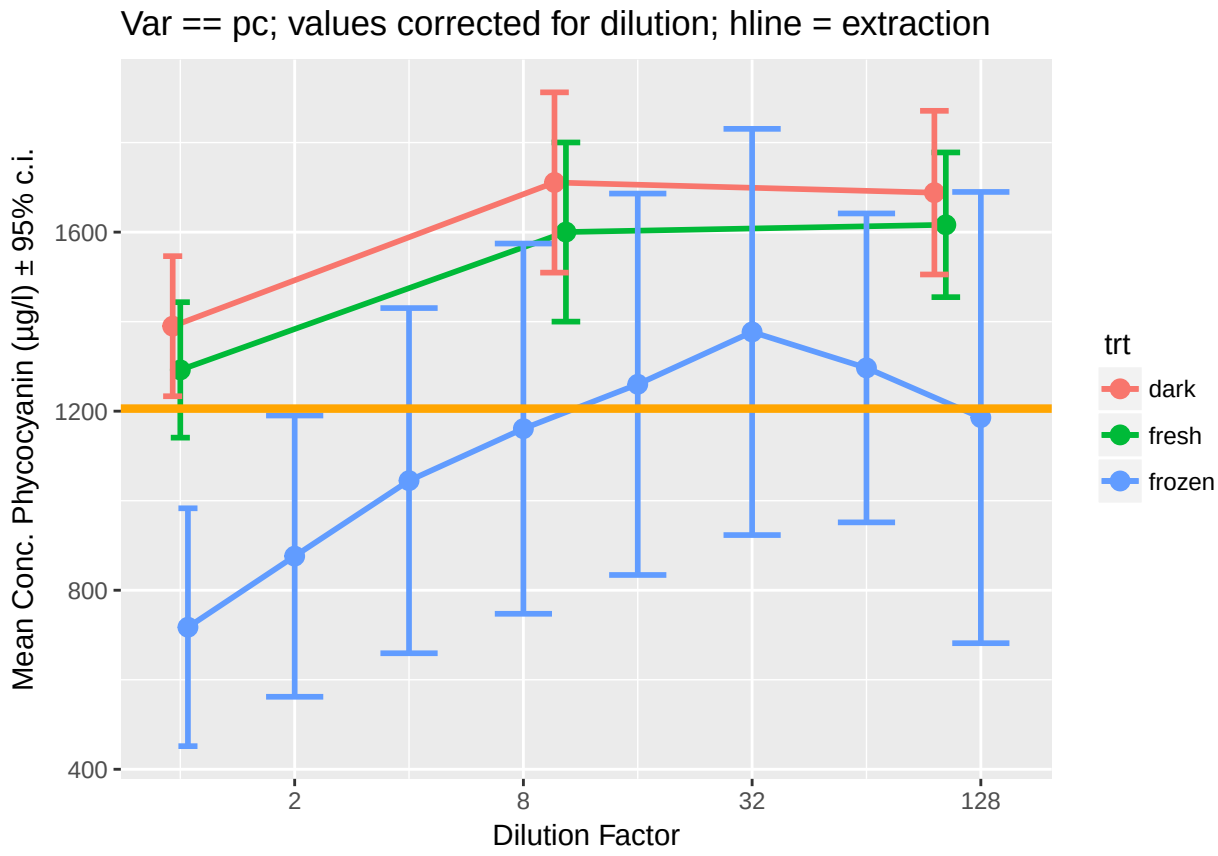


Fresh vs. Frozen vs Dark Adapted

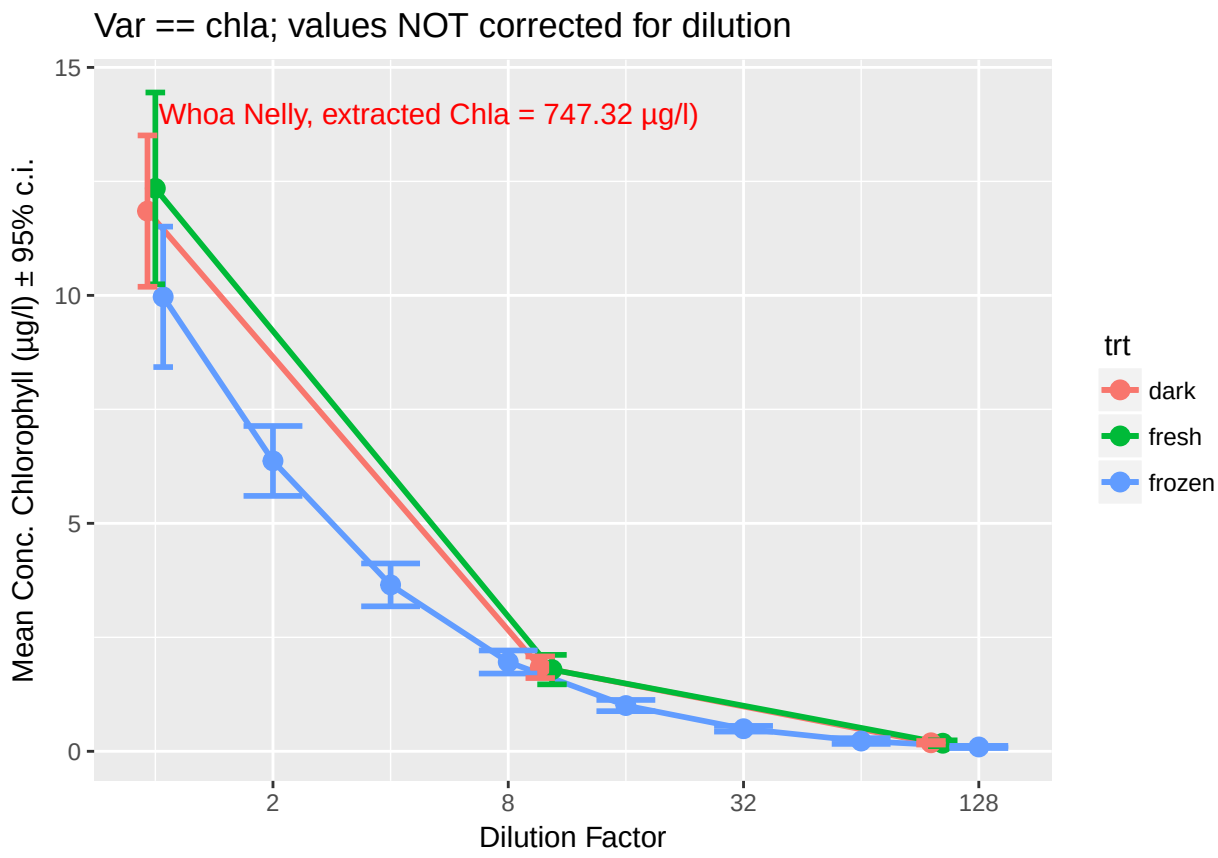
```
## Warning: position_dodge requires non-overlapping x intervals
```



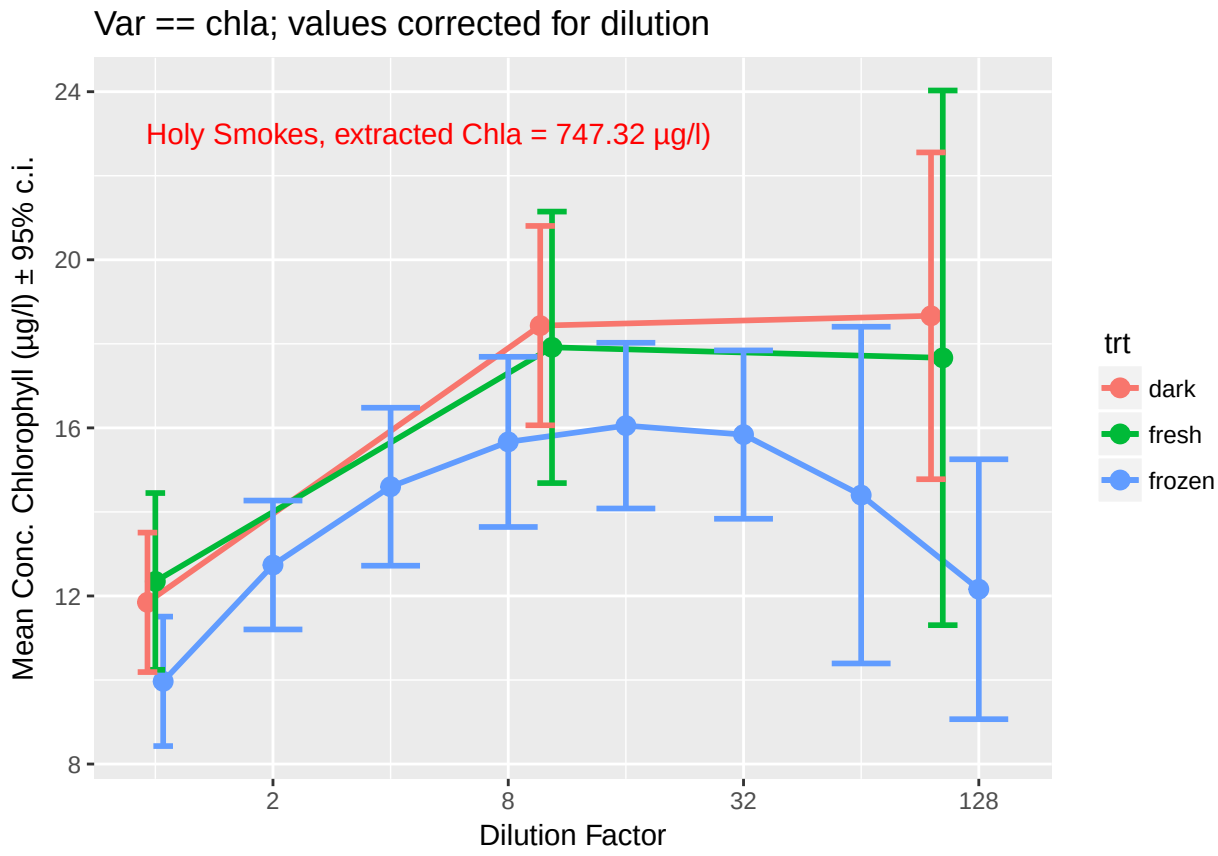

```
## Warning: position_dodge requires non-overlapping x intervals
```



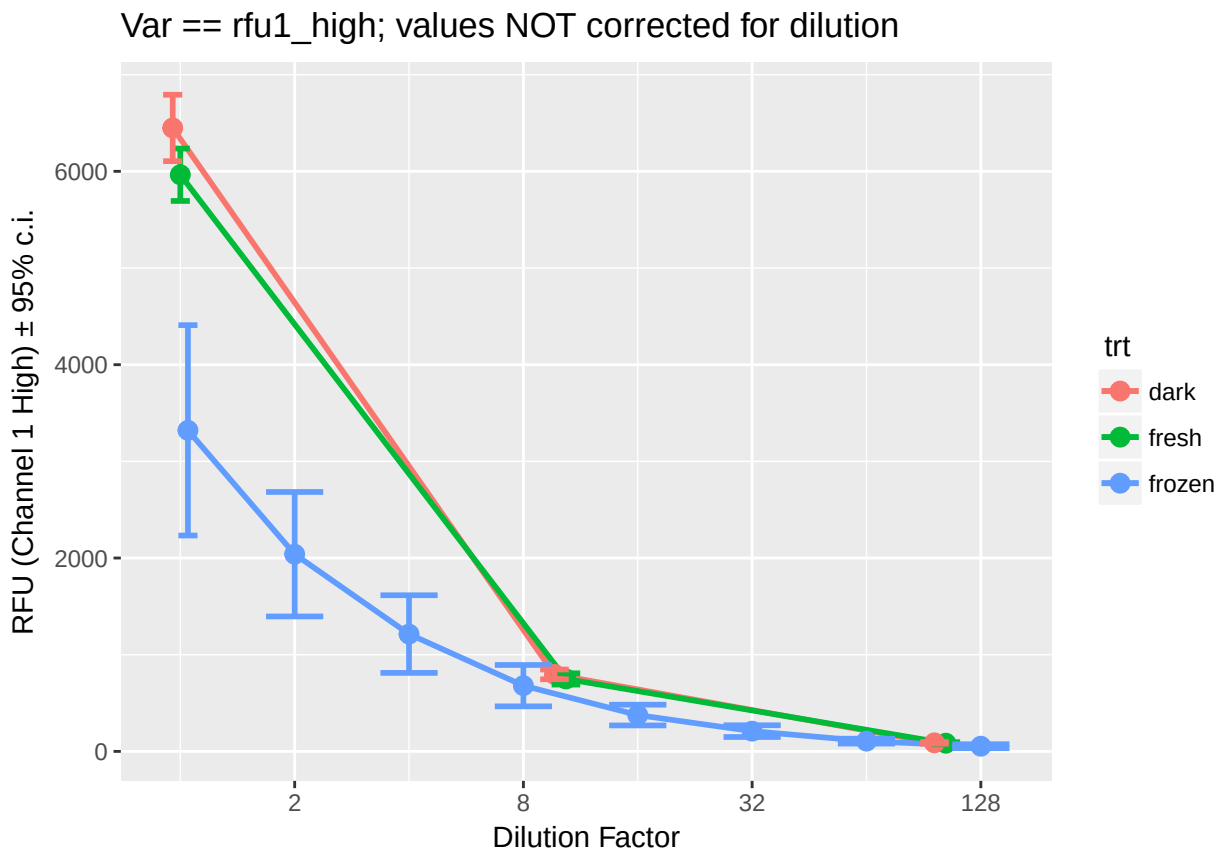

```
## Warning: position_dodge requires non-overlapping x intervals
```



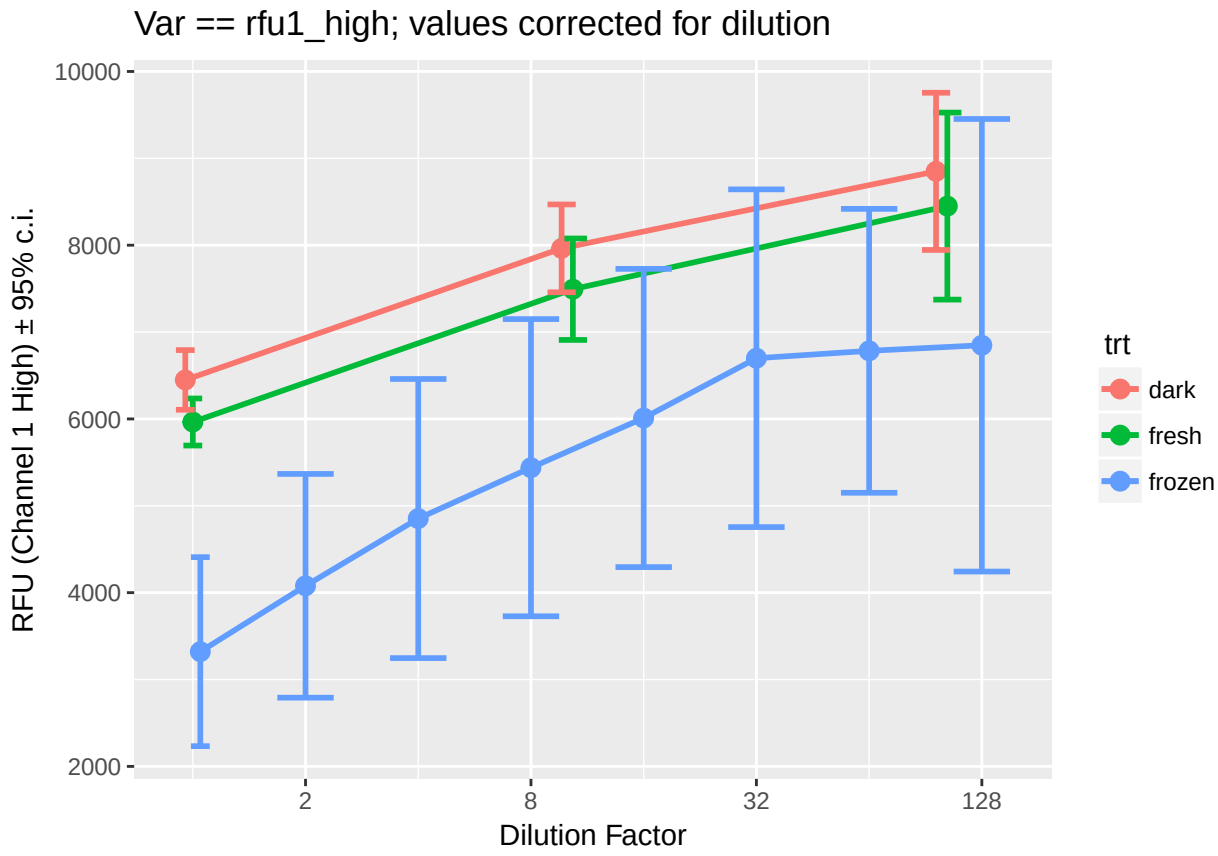

```
## Warning: position_dodge requires non-overlapping x intervals
```



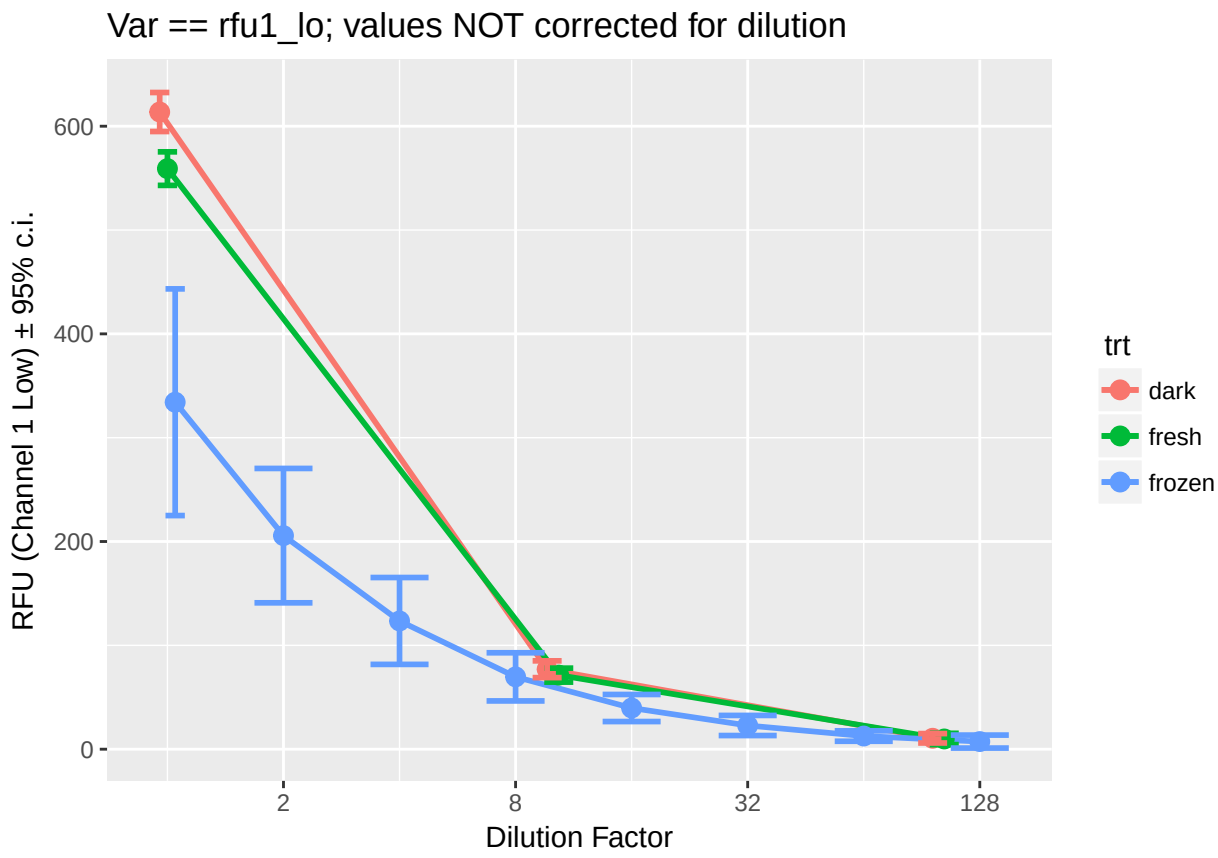

```
## Warning: position_dodge requires non-overlapping x intervals
```



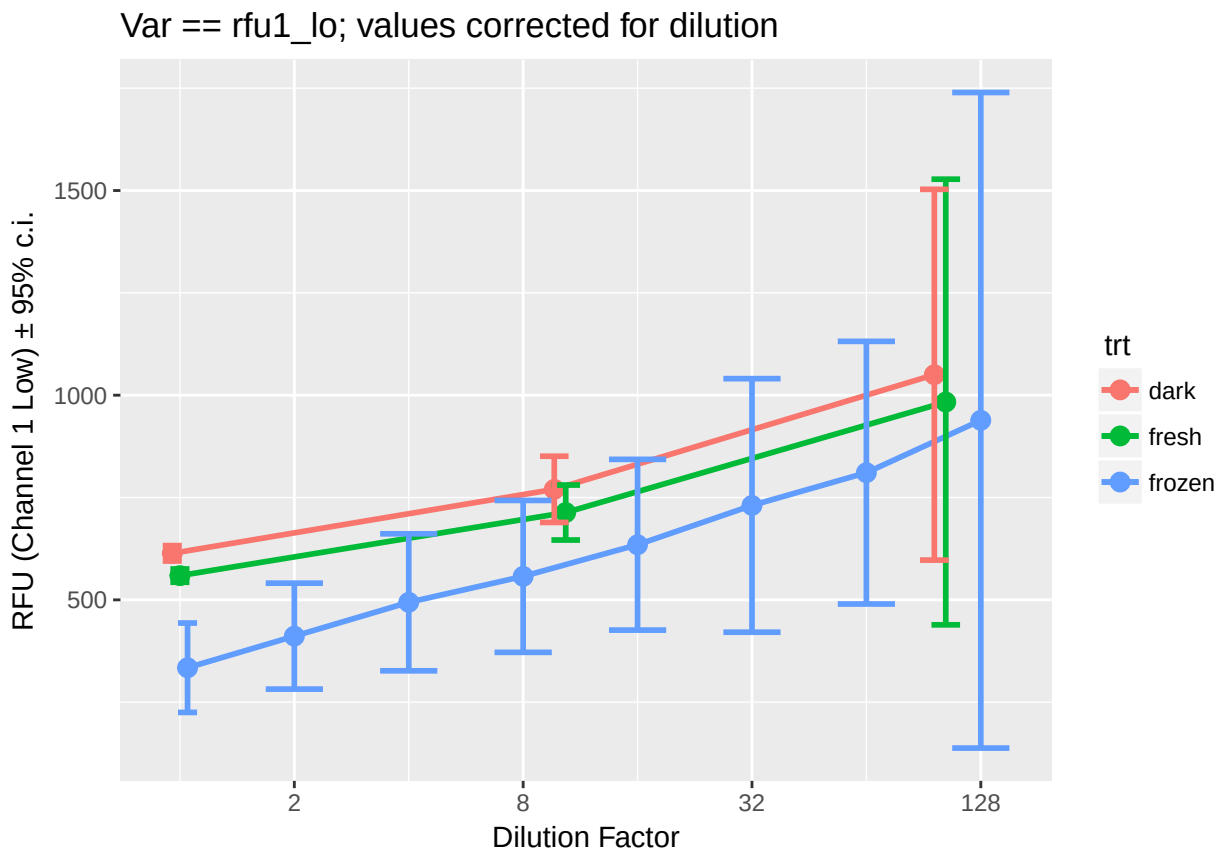

```
## Warning: position_dodge requires non-overlapping x intervals
```



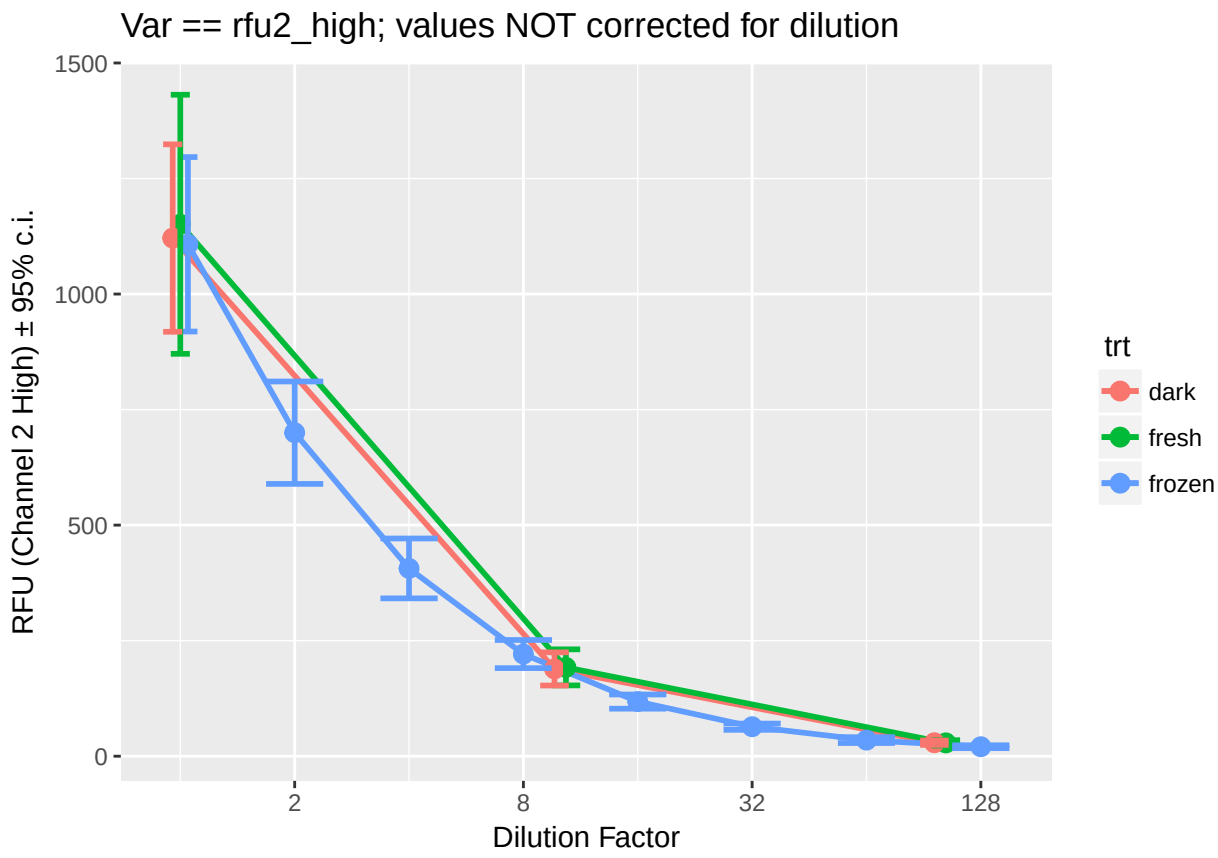

```
## Warning: position_dodge requires non-overlapping x intervals
```



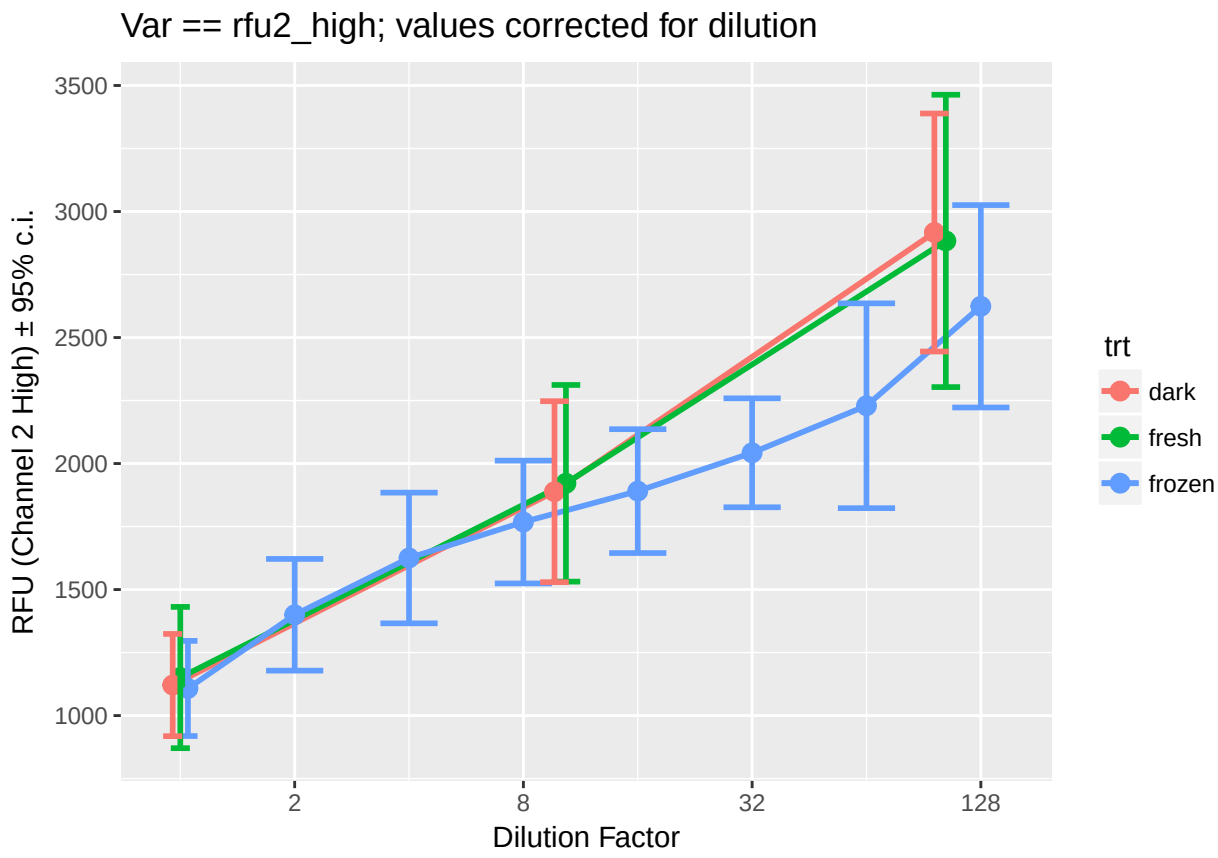

```
## Warning: position_dodge requires non-overlapping x intervals
```



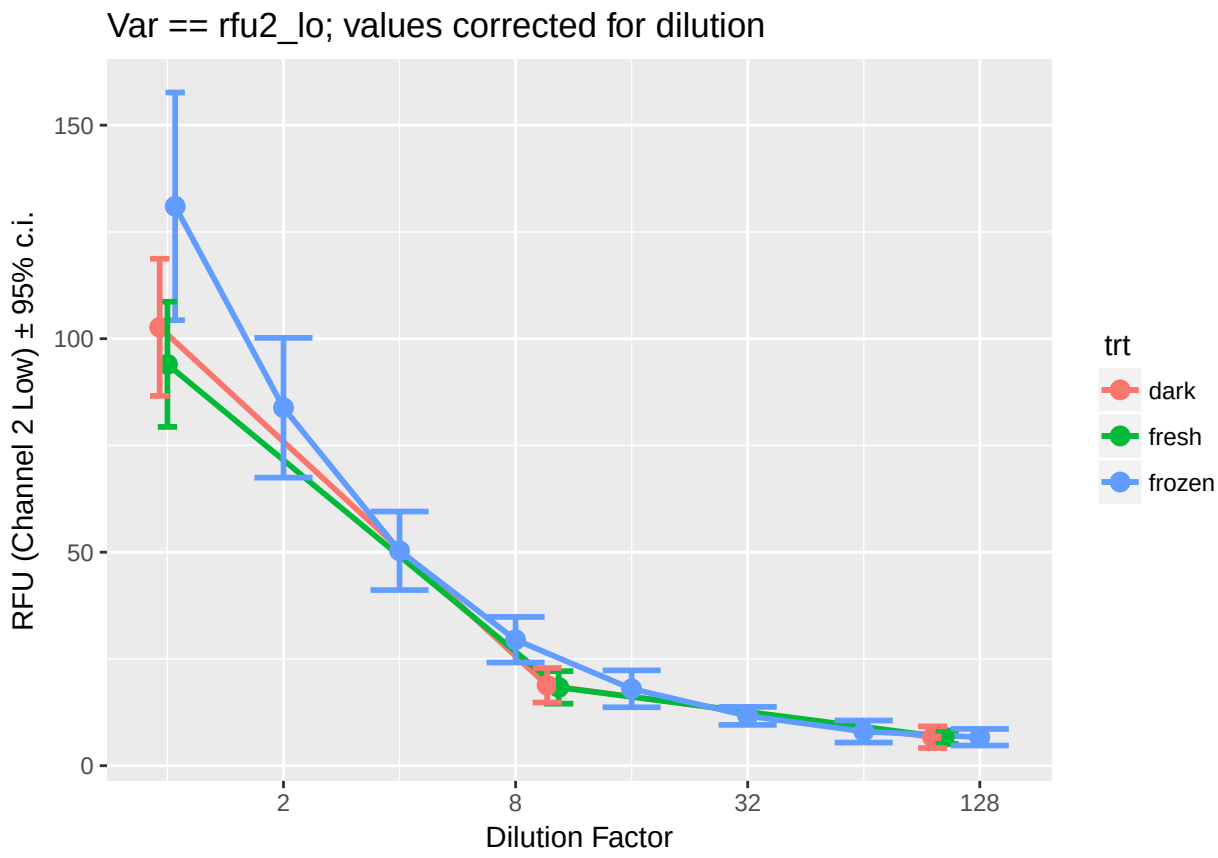

```
## Warning: position_dodge requires non-overlapping x intervals
```



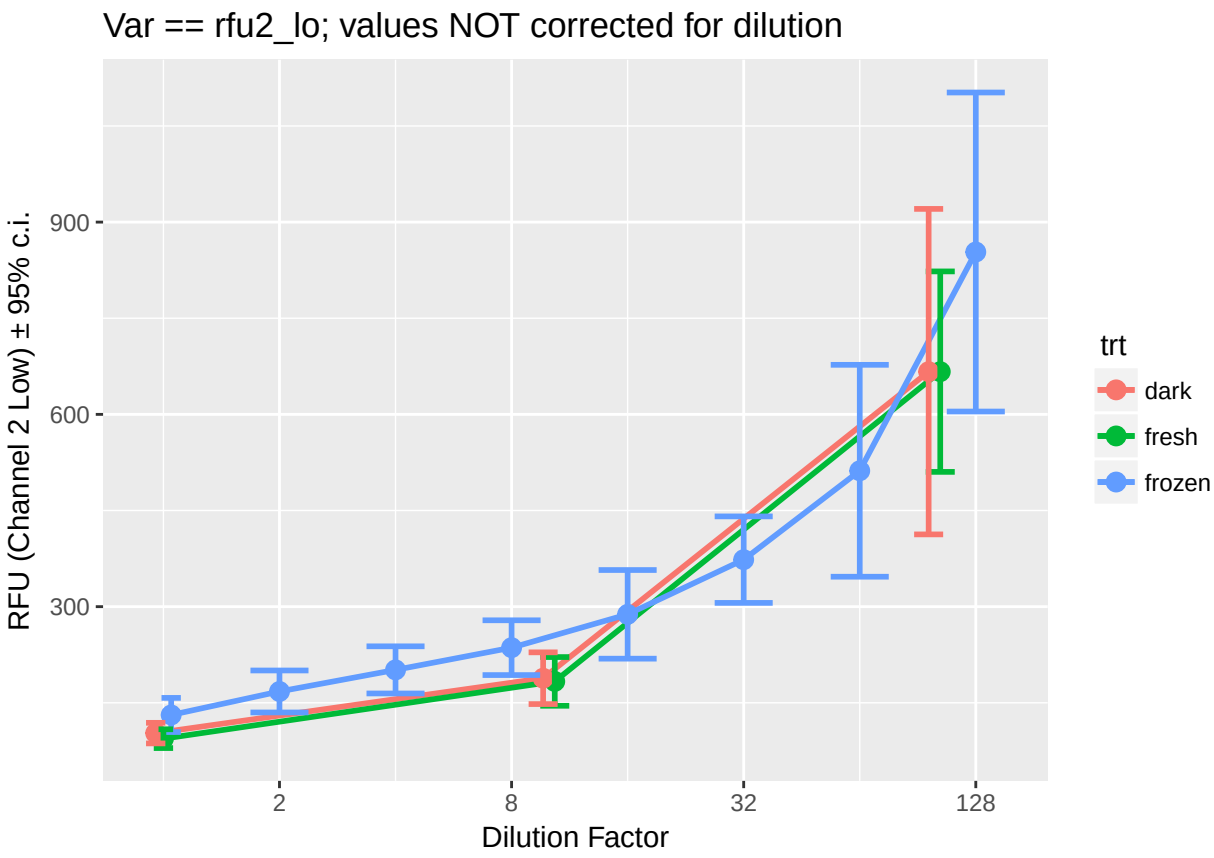

```
## Warning: position_dodge requires non-overlapping x intervals
```




```
## Warning: position_dodge requires non-overlapping x intervals
```




```
## Warning: position_dodge requires non-overlapping x intervals
```


Var == rfu2_lo; values NOT corrected for dilution
RFU (Channel 2 Low) ± 95% c.i.
900
600
300
2
8
32
128
Dilution Factor
trt
dark
fresh
frozen