Critical Oxygen Pressure (P_{CRIT}) and Oxygen Supply Capacity (α) Analysis

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2022-11-06

Contents

1 Loading Libraries

I am reading in the libraries I use for this analysis. Included among these is the "OTools" package, which was written by Kirt Onthank. This can be install from github using the command:

```
install_github('KirtOnthank/OTools')
```

```
library(OTools)
library(respirometry)
library(knitr)
library(nlme)
library(car)
library(emmeans)
```

2 Sorting files for P_{CRIT} analysis

```
files=list.files(recursive=T)
resp.files=grep(".txt",files,value=T)
pcrit.files=grep("pcrit|pcrti",resp.files,value=T,ignore.case=T)
pcrit.files=pcrit.files[!duplicated(basename(pcrit.files))]

pcrit.files.read=pcrit.files[!grepl("ch2.txt|ch3.txt|ch4.txt|\\(1\\).txt",pcrit.files)]
pcrit.files.read
```

```
##  [1] "All Pcrits/Gr1 Muus 1000-2 pcrit 7-27-21 B.txt"
##  [2] "All Pcrits/Gr1 Muus 1000-2 pcrit 7-27-21.txt"
##  [3] "All Pcrits/Gr1 Muus 1800-2 pcrit 25 ml jar 7-29-21 ch2 blank.txt"
##  [4] "All Pcrits/Gr1 Muus 1800-2 pcrit 7-28-21.txt"
##  [5] "All Pcrits/GR1 Muus 1800-2 pcrit day7 8-3-21.txt"
##  [6] "All Pcrits/GR1 Muus1000 7day-7-26-21.txt"
##  [7] "All Pcrits/GR1 Muus1000 pcrit 7-21-21.txt"
##  [8] "All Pcrits/GR1 Muus1800 7day-pcrit 7-20-21.txt"
##  [9] "All Pcrits/GR1 Muus1800 pcrit 7-13-21.txt"
## [10] "All Pcrits/gr2muus1800 7day pcrit 7-20-21.txt"
## [11] "All Pcrits/gr2muus1800-2 pcrit 7-28-21.txt"
## [12] "All Pcrits/gr2muus1800-2 pcrit day7 8-3-21.txt"
## [13] "All Pcrits/gr2muus1800-2 pcrit in 25 ml jar 7-29-21 ch2 is blank.txt"
## [14] "All Pcrits/Gr3 Muus 1000 pcrit 7-21-21.txt"
## [15] "All Pcrits/gr3 muus 1800 7day Pcrit 7-20-21.txt"
## [16] "All Pcrits/gr3 muus 1800 pcrit 7-13-21.txt"
## [17] "All Pcrits/Gr3 Muus 1800-2 pcrit 07-28-21.txt"
## [18] "All Pcrits/Gr3 Muus 1800-2 pcrit 08-03-21.txt"
## [19] "All Pcrits/Gr3 Muus1000-2 7 day pcrit 7-27-21.txt"
## [20] "All Pcrits/GR4MUUS1000-2Pcrit-7-26-21-ch1.txt"
## [21] "All Pcrits/GR4MUUS1000Pcrit-7-21-21-ch1.txt"
## [22] "All Pcrits/GR4MUUS1800-2-7dayPcrit-8-3-21-ch1.txt"
## [23] "All Pcrits/GR4MUUS1800-2Pcrit-7-28-21-ch1.txt"
## [24] "All Pcrits/GR4MUUS1800-7dayPcrit-7-20-21-ch1.txt"
## [25] "All Pcrits/GR4MUUS1800Pcrit-7-13-21-ch1.txt"
## [26] "All Pcrits/tbocto 1000 pcrit tank 1 and 2 day 7 8-19-21.txt"
```

```
## [27] "All Pcrits/tbocto 1000 pcrit tank 3 and 4 8-11-21-ch1.txt"
## [28] "All Pcrits/tbocto 1000 pcrit tank 3 and 4 day 7 8-19-21-ch1.txt"
## [29] "All Pcrits/Tbocto 1000 pcrti tank 1 and 2 8-11-21.txt"
## [30] "Group 2/Pcrit/gr2muus1000 pcrit 7-21-21.txt"
## [31] "Group 2/Pcrit/gr2muus1000-2 pcrit 7-26-21.txt"
## [32] "Trueblood after session/gr2MUUS1800-2pcritday7.8-3-21.txt"
## [33] "Trueblood after session/Muus TB collected data/desktop from presense onthank/tbocto 1800 pcrit
```

3 Reading in log files

Here I am reading in the log files that will provide additional information needed to analyze the raw data files.

```
pcrit.log=read.csv("pcrit_log.csv")
routine=read.csv("RMR_Results.csv")
```

The file named pcrit_log.csv contains all of the extra information that's needed to analyze the peak crip files exactly the way that we did. This includes the filenames in which the raw oxygen data is stored, the volume of the respirometer in which the analysis was performed in liters (vol), pCO2 in which the analysis was performed (pco2), the day of treatment on which the analysis was performed, where we trimmed the data to eliminate fluctuations at the beginning or the end of the trails (trim_left and trim_right), the identity of the octopus in the respirometer(s) (octo1 and octo2), and the file which contains the routine metabolic rate data which pairs with this analysis (rmr_match).

Here I am printing out the data in that file into tables to make it easier to view without opening up the CSV file in the directory. However, because of the many columns of data in that file, I need to print it out in four separate tables to make it format well in this PDF.

```
kable(pcrit.log[,1:6])
```

filename	ch1	ch2	vol	pco2	day
Gr1 Muus 1000-2 pcrit 7-27-21 B.txt	1	NA	1.000	1000	1
Gr1 Muus 1000-2 pcrit 7-27-21.txt	1	NA	1.000	1000	1
Gr1 Muus 1800-2 pcrit 25 ml jar 7-29-21 ch2 blank.txt	1	NA	0.025	1800	1
Gr1 Muus 1800-2 pcrit 7-28-21.txt	1	NA	0.120	1800	1
GR1 Muus 1800-2 pcrit day7 8-3-21.txt	1	NA	0.120	1800	7
GR1 Muus1000 7day-7-26-21.txt	1	NA	1.000	1000	7
GR1 Muus1000 pcrit 7-21-21.txt	1	NA	1.000	1000	1
GR1 Muus1800 7day-pcrit 7-20-21.txt	2	NA	1.000	1800	7
GR1 Muus1800 pcrit 7-13-21.txt	2	NA	1.000	1800	1
Gr2 Muus1000-2 pcrit 7-26-21.txt	1	NA	1.000	1000	1
gr2muus 1000 pcrit 7-21-21.txt	2	NA	1.000	1000	1
gr2muus1800 7day pcrit 7-20-21.txt	1	NA	1.000	1800	7
gr2muus1800-2 pcrit 7-28-21.txt	1	NA	1.000	1800	1
gr2muus1800-2 pcrit day7 8-3-21.txt	1	NA	1.000	1800	7
gr2muus1800-2 pcrit in 25 ml jar 7-29-21 ch2 is blank.txt	1	NA	0.025	1800	1
Gr3 Muus 1000 pcrit 7-21-21.txt	1	NA	1.000	1000	1
gr3 muus 1800 7day Pcrit 7-20-21.txt	1	NA	1.000	1800	7
gr3 muus 1800 pcrit 7-13-21.txt	1	NA	1.000	1800	1
Gr3 Muus 1800-2 pcrit 07-28-21.txt	1	NA	1.000	1800	1
Gr3 Muus 1800-2 pcrit 08-03-21.txt	1	NA	1.000	1800	7

filename	ch1	ch2	vol	pco2	day
Gr3 Muus1000-2 7 day pcrit 7-27-21.txt	1	NA	1.000	1000	7
GR4MUUS1000-2Pcrit-7-26-21-ch1.txt	1	NA	1.000	1000	7
GR4MUUS1000Pcrit-7-21-21-ch1.txt	1	NA	1.000	1000	1
GR4MUUS1800-7dayPcrit-7-20-21-ch1.txt	1	NA	1.000	1800	7
GR4MUUS1800-2-7dayPcrit-8-3-21-ch1.txt	1	NA	1.000	1800	7
GR4MUUS1800-2Pcrit-7-28-21-ch1.txt	1	NA	1.000	1800	1
GR4MUUS1800Pcrit-7-13-21-ch1.txt	1	NA	1.000	1800	1
tbocto 1000 pcrit tank 1 and 2 day 7 8-19-21.txt	1	3	1.000	1000	7
tbocto 1000 pcrit tank 3 and 4 8-11-21-ch1.txt	1	3	1.000	1000	1
tbocto 1000 pcrit tank 3 and 4 day 7 8-19-21-ch1.txt	1	3	1.000	1000	7
Tbocto 1000 pcrti tank 1 and 2 8-11-21.txt	1	3	1.000	1000	1
gr2muus1000 pcrit 7-21-21.txt	2	NA	1.000	1000	1
gr2muus1000-2 pcrit 7-26-21.txt	1	NA	1.000	1000	7
GR2MUUS18007dayPcrit-7-20-21.txt	2	NA	1.000	1800	7
gr2MUUS1800-2pcritday7.8-3-21.txt	1	NA	1.000	1800	7
tbocto 1800 pcrit day 7 tank 10 blank ch 3 4 8-20-21-ch1.txt	1	NA	1.000	1800	1

```
kable(pcrit.log[,c(1,7:10)])
```

filename	trim_left	trim_left2	trim_left3	trim_left4
Gr1 Muus 1000-2 pcrit 7-27-21 B.txt	0	0	NA	NA
Gr1 Muus 1000-2 pcrit 7-27-21.txt	0	0	NA	NA
Gr1 Muus 1800-2 pcrit 25 ml jar 7-29-21 ch2 blank.txt	2900	NA	NA	NA
Gr1 Muus 1800-2 pcrit 7-28-21.txt	3000	3000	NA	NA
GR1 Muus 1800-2 pcrit day7 8-3-21.txt	0	0	NA	NA
GR1 Muus1000 7day-7-26-21.txt	32000	32000	NA	NA
GR1 Muus1000 pcrit 7-21-21.txt	100	100	NA	NA
GR1 Muus1800 7day-pcrit 7-20-21.txt	1000	1000	NA	NA
GR1 Muus1800 pcrit 7-13-21.txt	500	0	NA	NA
Gr2 Muus1000-2 pcrit 7-26-21.txt	0	0	NA	NA
gr2muus 1000 pcrit 7-21-21.txt	200	0	NA	NA
gr2muus1800 7day pcrit 7-20-21.txt	0	0	NA	NA
gr2muus1800-2 pcrit 7-28-21.txt	2000	2000	NA	NA
gr2muus1800-2 pcrit day7 8-3-21.txt	0	0	NA	NA
gr2muus1800-2 pcrit in 25 ml jar 7-29-21 ch2 is blank.txt	0	NA	NA	NA
Gr3 Muus 1000 pcrit 7-21-21.txt	0	0	NA	NA
gr3 muus 1800 7day Pcrit 7-20-21.txt	0	0	NA	NA
gr3 muus 1800 pcrit 7-13-21.txt	0	0	NA	NA
Gr3 Muus 1800-2 pcrit 07-28-21.txt	500	0	NA	NA
Gr3 Muus 1800-2 pcrit 08-03-21.txt	200	0	NA	NA
Gr3 Muus1000-2 7 day pcrit 7-27-21.txt	0	0	NA	NA
GR4MUUS1000-2Pcrit-7-26-21-ch1.txt	100	0	NA	NA
GR4MUUS1000Pcrit-7-21-21-ch1.txt	500	500	NA	NA
GR4MUUS1800-7dayPcrit-7-20-21-ch1.txt	0	1000	NA	NA
GR4MUUS1800-2-7dayPcrit-8-3-21-ch1.txt	0	0	NA	NA
GR4MUUS1800-2Pcrit-7-28-21-ch1.txt	200	200	NA	NA
GR4MUUS1800Pcrit-7-13-21-ch1.txt	8000	8000	NA	NA
tbocto 1000 pcrit tank 1 and 2 day 7 8-19-21.txt	8000	8000	0	0
tbocto 1000 pcrit tank 3 and 4 8-11-21-ch1.txt	500	500	200	200
tbocto 1000 pcrit tank 3 and 4 day 7 8-19-21-ch1.txt	0	0	0	0

filename	trim_left	trim_left2	trim_left3	trim_left4
Tbocto 1000 pcrti tank 1 and 2 8-11-21.txt	1000	1000	200	200
gr2muus1000 pcrit 7-21-21.txt	100	0	NA	NA
gr2muus1000-2 pcrit 7-26-21.txt	0	0	NA	NA
GR2MUUS18007dayPcrit-7-20-21.txt	0	0	NA	NA
gr2MUUS1800-2pcritday7.8-3-21.txt	0	0	NA	NA
tbocto 1800 pcrit day 7 tank 10 blank ch 3 4 8-20-21-ch1.txt	200	200	NA	NA

```
kable(pcrit.log[,c(1,11:14)])
```

filename	trim_right	trim_right2	trim_right3	trim_right4
Gr1 Muus 1000-2 pcrit 7-27-21 B.txt	0	0	NA	NA
Gr1 Muus 1000-2 pcrit 7-27-21.txt	0	0	NA	NA
Gr1 Muus 1800-2 pcrit 25 ml jar 7-29-21 ch2 blank.txt	200	NA	NA	NA
Gr1 Muus 1800-2 pcrit 7-28-21.txt	4000	4000	NA	NA
GR1 Muus 1800-2 pcrit day7 8-3-21.txt	0	0	NA	NA
GR1 Muus1000 7day-7-26-21.txt	20000	20000	NA	NA
GR1 Muus1000 pcrit 7-21-21.txt	300	300	NA	NA
GR1 Muus1800 7day-pcrit 7-20-21.txt	1000	1000	NA	NA
GR1 Muus1800 pcrit 7-13-21.txt	0	0	NA	NA
Gr2 Muus1000-2 pcrit 7-26-21.txt	0	0	NA	NA
gr2muus 1000 pcrit 7-21-21.txt	0	0	NA	NA
gr2muus1800 7day pcrit 7-20-21.txt	0	0	NA	NA
gr2muus1800-2 pcrit 7-28-21.txt	4000	2000	NA	NA
gr2muus1800-2 pcrit day7 8-3-21.txt	0	0	NA	NA
gr2muus1800-2 pcrit in 25 ml jar 7-29-21 ch2 is blank.txt	2800	NA	NA	NA
Gr3 Muus 1000 pcrit 7-21-21.txt	4600	4600	NA	NA
gr3 muus 1800 7day Pcrit 7-20-21.txt	0	200	NA	NA
gr3 muus 1800 pcrit 7-13-21.txt	0	0	NA	NA
Gr3 Muus 1800-2 pcrit 07-28-21.txt	0	0	NA	NA
Gr3 Muus 1800-2 pcrit 08-03-21.txt	100	100	NA	NA
Gr3 Muus1000-2 7 day pcrit 7-27-21.txt	0	0	NA	NA
GR4MUUS1000-2Pcrit-7-26-21-ch1.txt	0	0	NA	NA
GR4MUUS1000Pcrit-7-21-21-ch1.txt	4400	4400	NA	NA
GR4MUUS1800-7dayPcrit-7-20-21-ch1.txt	0	0	NA	NA
GR4MUUS1800-2-7dayPcrit-8-3-21-ch1.txt	14000	14000	NA	NA
GR4MUUS1800-2Pcrit-7-28-21-ch1.txt	100	100	NA	NA
GR4MUUS1800Pcrit-7-13-21-ch1.txt	0	0	NA	NA
tbocto 1000 pcrit tank 1 and 2 day 7 8-19-21.txt	5500	5500	18000	18000
tbocto 1000 pcrit tank 3 and 4 8-11-21-ch1.txt	200	200	14800	14800
tbocto 1000 pcrit tank 3 and 4 day 7 8-19-21-ch1.txt	200	0	200	200
Tbocto 1000 pcrti tank 1 and 2 8-11-21.txt	0	0	7000	7000
gr2muus1000 pcrit 7-21-21.txt	0	0	NA	NA
gr2muus1000-2 pcrit 7-26-21.txt	0	0	NA	NA
GR2MUUS18007dayPcrit-7-20-21.txt	0	0	NA	NA
gr2MUUS1800-2pcritday7.8-3-21.txt	5000	5000	NA	NA
tbocto 1800 pcrit day 7 tank 10 blank ch 3 4 8-20-21-ch1.txt	0	0	NA	NA

```
kable(pcrit.log[,c(1,15:17)])
```

filename	octo1	octo2	rmr_match
Gr1 Muus 1000-2 pcrit 7-27-21 B.txt	1-2		GR1 Muus1000 7day-7-26-21.txt
Gr1 Muus 1000-2 pcrit 7-27-21.txt	1-2		GR1 Muus1000 7day-7-26-21.txt
Gr1 Muus 1800-2 pcrit 25 ml jar 7-29-21 ch2 blank.txt	1-3		Gr1 Muus 1800-2 7day-8-2-21.txt
Gr1 Muus 1800-2 pcrit 7-28-21.txt	1-3		Gr1 Muus 1800-2 7day-8-2-21.txt
GR1 Muus 1800-2 pcrit day7 8-3-21.txt	1-3		Gr1 Muus 1800-2 7day-8-2-21.txt
GR1 Muus1000 7day-7-26-21.txt	1-2		GR1 Muus1000 7day-7-26-21.txt
GR1 Muus1000 pcrit 7-21-21.txt	1-2		GR1 Muus1000 7-20-21.txt
GR1 Muus1800 7day-pcrit 7-20-21.txt	1-1		GR1 Muus1800 7day-7-19-21.txt
GR1 Muus1800 pcrit 7-13-21.txt	1-1		GR1 Muus1800 7-12-21.txt
Gr2 Muus1000-2 pcrit 7-26-21.txt	2-2		gr2muus1000-2 7-26-21.txt
gr2muus 1000 pcrit 7-21-21.txt	2-2		gr2muus1000 7-20-21.txt
gr2muus1800 7day pcrit 7-20-21.txt	2-1		gr2muus1800 7day 7-19-21.txt
gr2muus1800-2 pcrit 7-28-21.txt	2-3		gr2muus1800-2 7-27-21 forgot to start log.txt
gr2muus1800-2 pcrit day7 8-3-21.txt	2-3		gr2muus1800-2 day7 8-2-21.txt
gr2muus1800-2 pcrit in 25 ml jar 7-29-21 ch2 is blank.txt	2-3		gr2muus1800-2 day7 8-2-21.txt
Gr3 Muus 1000 pcrit 7-21-21.txt	3-2		gr3 muus 1000 7-20-21.txt
gr3 muus 1800 7day Pcrit 7-20-21.txt	3-1		gr3 muus 1800 7day-7-19-21.txt
gr3 muus 1800 pcrit 7-13-21.txt	3-1		gr3 muus 1800 7-12-21.txt
Gr3 Muus 1800-2 pcrit 07-28-21.txt	3-3		gr3 muus 1800 -2 7-27-21.txt
Gr3 Muus 1800-2 pcrit 08-03-21.txt	3-3		gr3 muus 1800 -2 day7 8-02-21.txt
Gr3 Muus1000-2 7 day pcrit 7-27-21.txt	3-2		gr3 muus 1000-2 7day 7-26-21.txt
GR4MUUS1000-2Pcrit-7-26-21-ch1.txt	4-2		
GR4MUUS1000Pcrit-7-21-21-ch1.txt	4-2		GR4MUUS1000-7-21-21-ch1.txt
GR4MUUS1800-7dayPcrit-7-20-21-ch1.txt	4-1		GR4MUUS1800-7day-7-19-21.txt
GR4MUUS1800-2-7dayPcrit-8-3-21-ch1.txt	4-3		GR4MUUS1800-2-7day-8-2-21.txt
GR4MUUS1800-2Pcrit-7-28-21-ch1.txt	4-3		
GR4MUUS1800Pcrit-7-13-21-ch1.txt	4-1		GR4MUUS18007.12.21MUUS-ch1
tbocto 1000 pcrit tank 1 and 2 day 7 8-19-21.txt	5-1	5-2	tbocto 1000 rmr tank 1 and 2 8-10-21 1 ch 1-2 2 ch3-4.txt
tbocto 1000 pcrit tank 3 and 4 8-11-21-ch1.txt	5-3	5-4	
tbocto 1000 pcrit tank 3 and 4 day 7 8-19-21-ch1.txt	5-3	5-4	tbocto 1000 rmr tank 3 and 4 day 7 8-18-21-ch1.txt
Tbocto 1000 pcrti tank 1 and 2 8-11-21.txt	5-1	5-2	tbocto 1000 rmr tank 1 and 2 8-10-21 1 ch 1-2 2 ch3-4.txt
gr2muus1000 pcrit 7-21-21.txt	2-2		gr2muus1000 7-20-21.txt
gr2muus1000-2 pcrit 7-26-21.txt	2-2		gr2muus1000-2 7-26-21.txt
GR2MUUS18007dayPcrit-7-20-21.txt	2-1		gr2muus1800 7day 7-19-21.txt
gr2MUUS1800-2pcritday7.8-3-21.txt	2-3		gr2muus1800-2 day7 8-2-21.txt
tbocto 1800 pcrit day 7 tank 10 blank ch 3 4 8-20-21-ch1.txt	5-5		unknown

4 Calculating P_{CRIT} from raw data

First I make a empty object where I can place calculated P_{CRIT}s

```
pcrits=data.frame(filename=as.character(),
                  spreadsheet_guess=as.character(),
                  octo=as.character(),
                  mass=as.numeric(),
                  pco2=as.numeric(),
                  day=as.numeric(),
                  rmr=as.numeric(),
                  pcrit=as.numeric(),
                  alpha=as.numeric()
                  )
```

Next, I calculate the P_{CRIT} from each data file. Here are a couple of important points on our calculations:

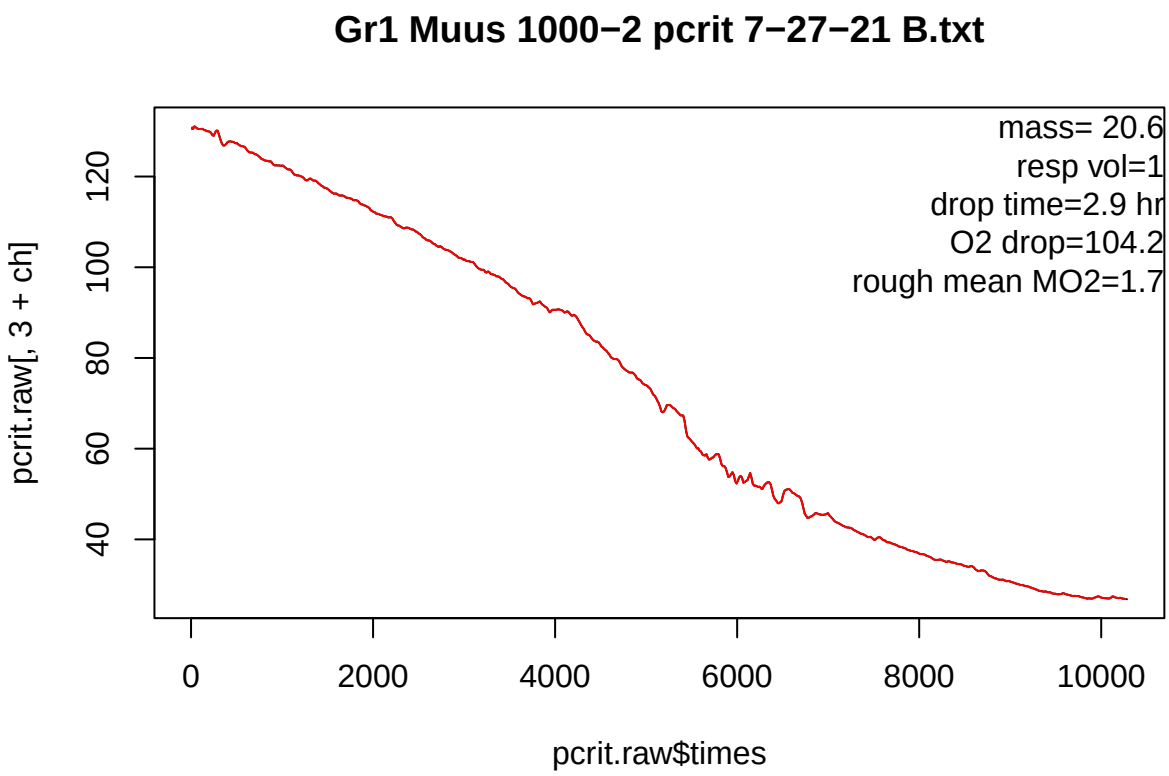
1. We are using the alpha P_{CRIT} method (Seibel et al, 2021) to calculate P_{CRIT} as implemented by the calc_pcrit() function from the "respirometry" R package (Birk, 2021).
2. We used the routine metabolic rate that we measured for each individual octopus in this study, unless the maximum metabolic rate measured during the P_{CRIT} run was less than the RMR we had measured. In those cases we used the default MR used by the function, which is "the mean MO2 value from the oxyregulating portion of the curve (as defined by the broken-stick regression)".
3. Any run in which O_2 did not drop below 50 $mmHgO_2$ was dropped from the analysis. All runs, however, including those dropped, are graphed below.
4. Oxygen trace of each run is graphed below with the portion used to calculate P_{CRIT} plotted in red.

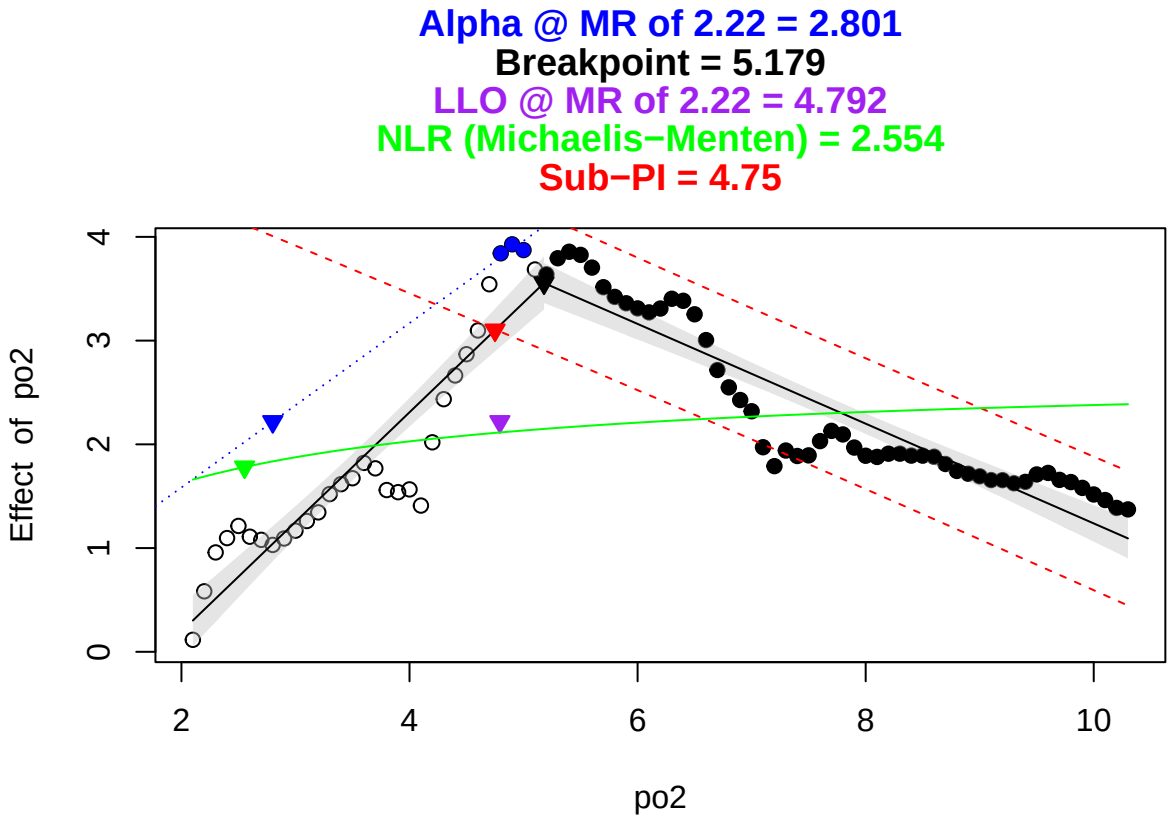
```
co=1

for (i in 1:length(pcrit.files.read)){
  filename=pcrit.files.read[i]

  if(length(grep("Group 4|presens|ch\\d\\.txt",basename(filename)))>0){
    pcrit.raw=read.presens(filename)
  }else{
    pcrit.raw=read.pyro(filename)
  }

guess=which.min(adist(basename(filename),pcrit.log$filename))
ch=pcrit.log$ch1[guess]
octo=pcrit.log$octo1[guess]
start=pcrit.log[guess,6+ch]
stop=max(pcrit.raw$times)-pcrit.log[guess,10+ch]
mass=mean(routine$mass[routine$octo==octo])
rmr=mean(routine$rmr[routine$octo==octo])

pcrit.working=
  pcrit.raw[
    pcrit.raw$times>start&
    pcrit.raw$times<stop,
  ]
vol=pcrit.log$vol[guess]
drop.time=round((stop-start)/3600,1)
O2.drop=round(diff(range(pcrit.working[,3+ch])),1)
rough.mo2=round((O2.drop*vol)/mass/drop.time,1)

plot(pcrit.raw[,3+ch]~pcrit.raw$times,type="l",main=basename(filename))
```

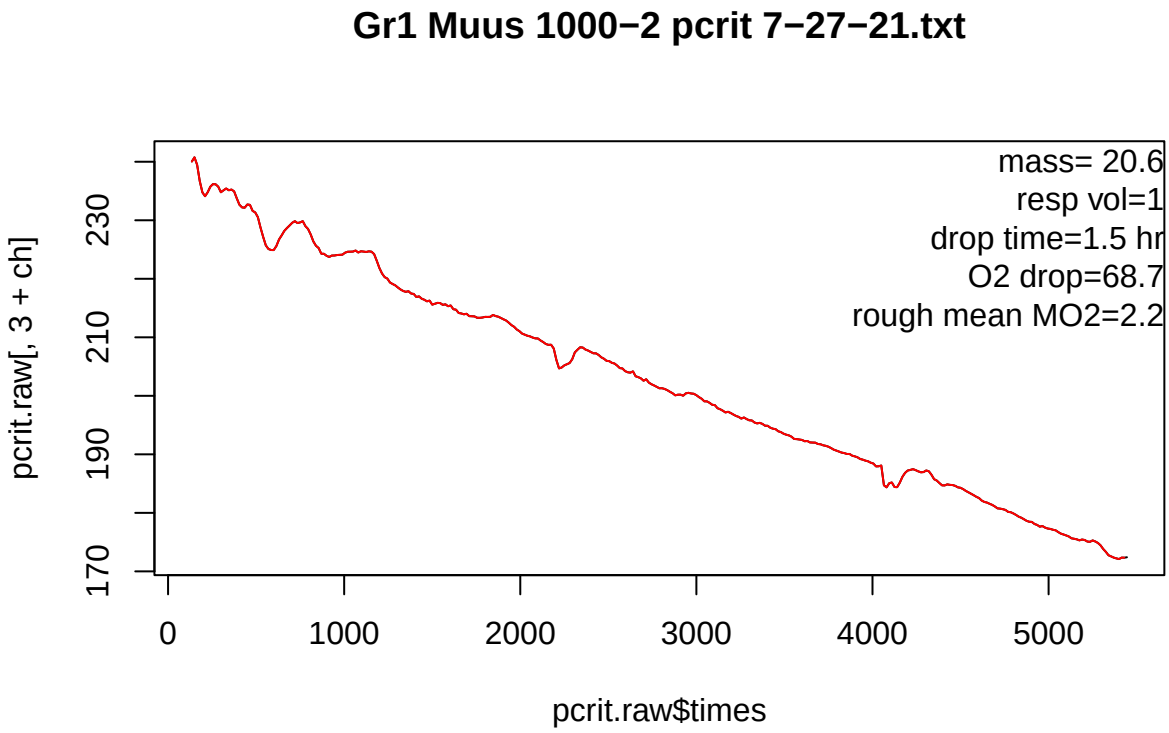
```
points(pcrit.working[,3+ch]~pcrit.working$times,type="l",col="red")
mtext(paste("mass=",mass),side=3,adj=1,line=-1)
mtext(paste0("resp vol=",vol),side=3,adj=1,line=-2)
mtext(paste0("drop time=",drop.time," hr"),side=3,adj=1,line=-3)
mtext(paste0("O2 drop=",O2.drop),side=3,adj=1,line=-4)
mtext(paste0("rough mean MO2=",rough.mo2),side=3,adj=1,line=-5)

pcrit.resp=resp.closed(pcrit.working,volume=pcrit.log$vol[guess],
            weight=mass,smooth="loess",channel=ch,smooth.span = 0.2)

pcrit.bin=aggregate(pcrit.resp$resp~round(pcrit.resp$po2,1),FUN="mean")
colnames(pcrit.bin)=c("po2","resp")

if(max(pcrit.bin$resp)>rmr){
  plot_pcrit(pcrit.bin$po2,pcrit.bin$resp,
           avg_top_n = 3,MR=rmr)
  if (min(pcrit.working[,3+ch])>50){
      mtext("DROPPED FROM ANALYSIS",line=-5,col="red",cex=3)
      mtext("pO2 did not get low enough",line=-7,col="red",cex=2)
    }
}else{
  plot_pcrit(pcrit.bin$po2,pcrit.bin$resp,
           avg_top_n = 3)
  if (min(pcrit.working[,3+ch])>50){
      mtext("DROPPED FROM ANALYSIS",line=-5,col="red",cex=3)
      mtext("pO2 did not get low enough",line=-7,col="red",cex=2)
    }
}


pcrits[co,1]=basename(filename)
pcrits[co,2]=pcrit.log$filename[guess]
pcrits[co,3]=octo
pcrits[co,4]=mass
pcrits[co,5]=pcrit.log$pco2[guess]
pcrits[co,6]=pcrit.log$day[guess]
pcrits[co,7]=rmr
if (min(pcrit.working[,3+ch])<50){
  if(max(pcrit.bin$resp)>rmr){
  pcrits[co,8]=as.numeric(calc_pcrit(pcrit.bin$po2,pcrit.bin$resp,
           avg_top_n = 3,MR=rmr)[1])
  }else{
    pcrits[co,8]=as.numeric(calc_pcrit(pcrit.bin$po2,pcrit.bin$resp,
           avg_top_n = 3)[1])
  }
  pcrits[co,9]=calc_alpha(pcrit.bin$po2,pcrit.bin$resp,avg_top_n = 3)$alpha
}else{
  pcrits[co,8]=NA
  pcrits[co,9]=NA
}
co=co+1

if(!is.na(pcrit.log$ch2[guess])){
```

```
ch=pcrit.log$ch2[guess]
octo=pcrit.log$octo2[guess]
start=pcrit.log[guess,6+ch]
stop=max(pcrit.raw$times)-pcrit.log[guess,10+ch]
mass=mean(routine$mass[routine$octo==octo])
rmr=mean(routine$rmr[routine$octo==octo])

pcrit.working=
  pcrit.raw[
    pcrit.raw$times>start&
    pcrit.raw$times<stop,
  ]

plot(pcrit.raw[,3+ch]~pcrit.raw$times,type="l",main=basename(filename))
points(pcrit.working[,3+ch]~pcrit.working$times,type="l",col="red")

pcrit.resp=resp.closed(pcrit.working,volume=pcrit.log$vol[guess],
          weight=mass,smooth="loess",channel=ch,smooth.span = 0.2)
pcrit.bin=aggregate(pcrit.resp$resp~round(pcrit.resp$po2,1),FUN="mean")
colnames(pcrit.bin)=c("po2","resp")

if(max(pcrit.bin$resp)>rmr){
  plot_pcrit(pcrit.bin$po2,pcrit.bin$resp,
         avg_top_n = 3,MR=rmr)
  if (min(pcrit.working[,3+ch])>50){
    mtext("DROPPED FROM ANALYSIS",line=-5,col="red",cex=3)
    mtext("pO2 did not get low enough",line=-7,col="red",cex=2)
  }
}else{
  plot_pcrit(pcrit.bin$po2,pcrit.bin$resp,
         avg_top_n = 3)
  if (min(pcrit.working[,3+ch])>50){
    mtext("DROPPED FROM ANALYSIS",line=-5,col="red",cex=3)
    mtext("pO2 did not get low enough",line=-7,col="red",cex=2)
  }
}


pcrits[co,1]=basename(filename)
pcrits[co,2]=pcrit.log$filename[guess]
pcrits[co,3]=octo
pcrits[co,4]=mass
pcrits[co,5]=pcrit.log$pco2[guess]
pcrits[co,6]=pcrit.log$day[guess]
pcrits[co,7]=rmr
if (min(pcrit.working[,3+ch])<50){
  if(max(pcrit.bin$resp)>rmr){
  pcrits[co,8]=as.numeric(calc_pcrit(pcrit.bin$po2,pcrit.bin$resp,
         avg_top_n = 3,MR=rmr)[1])
  }else{
    pcrits[co,8]=as.numeric(calc_pcrit(pcrit.bin$po2,pcrit.bin$resp,
         avg_top_n = 3)[1])
  }
```

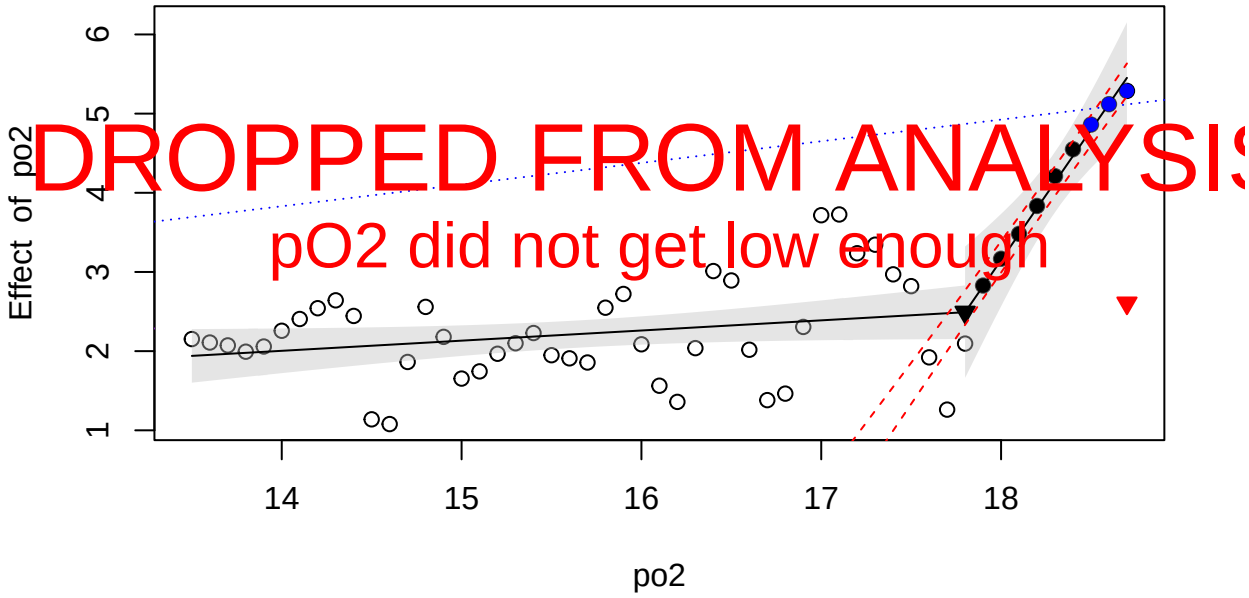
```
    pcrits[co,9]=calc_alpha(pcrit.bin$po2,pcrit.bin$resp,avg_top_n = 3)$alpha
  }else{
    pcrits[co,8]=NA
  }
  co=co+1

}


}
```

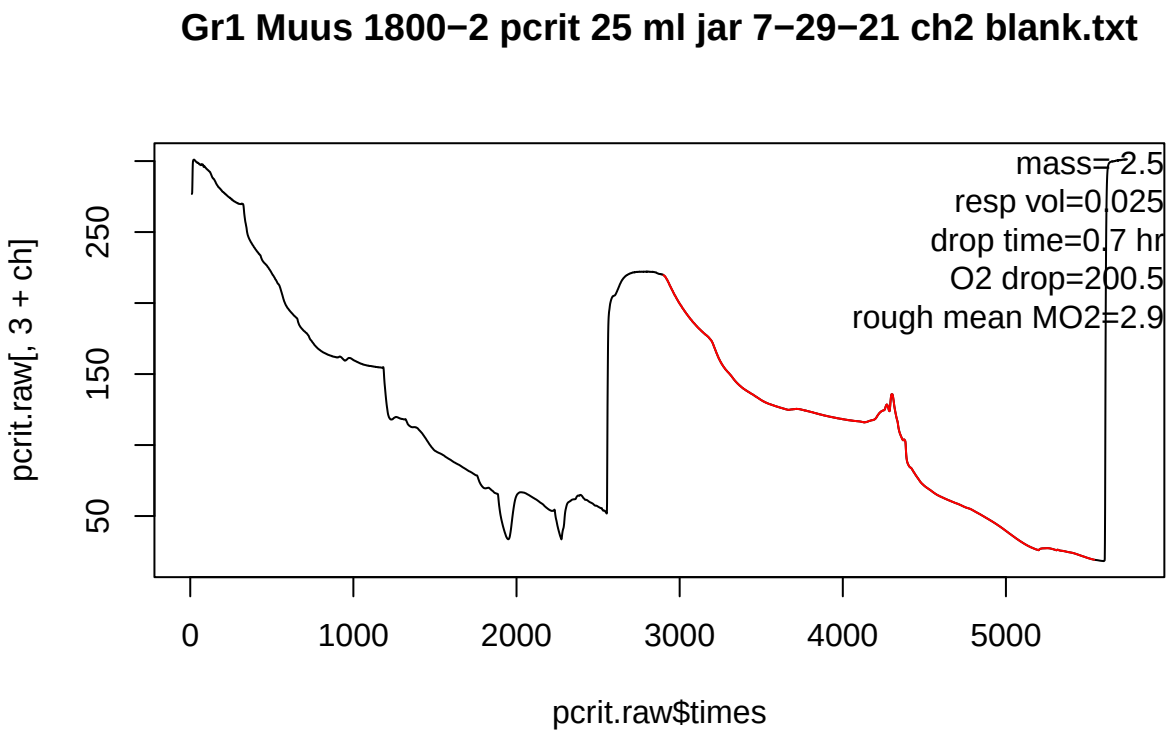


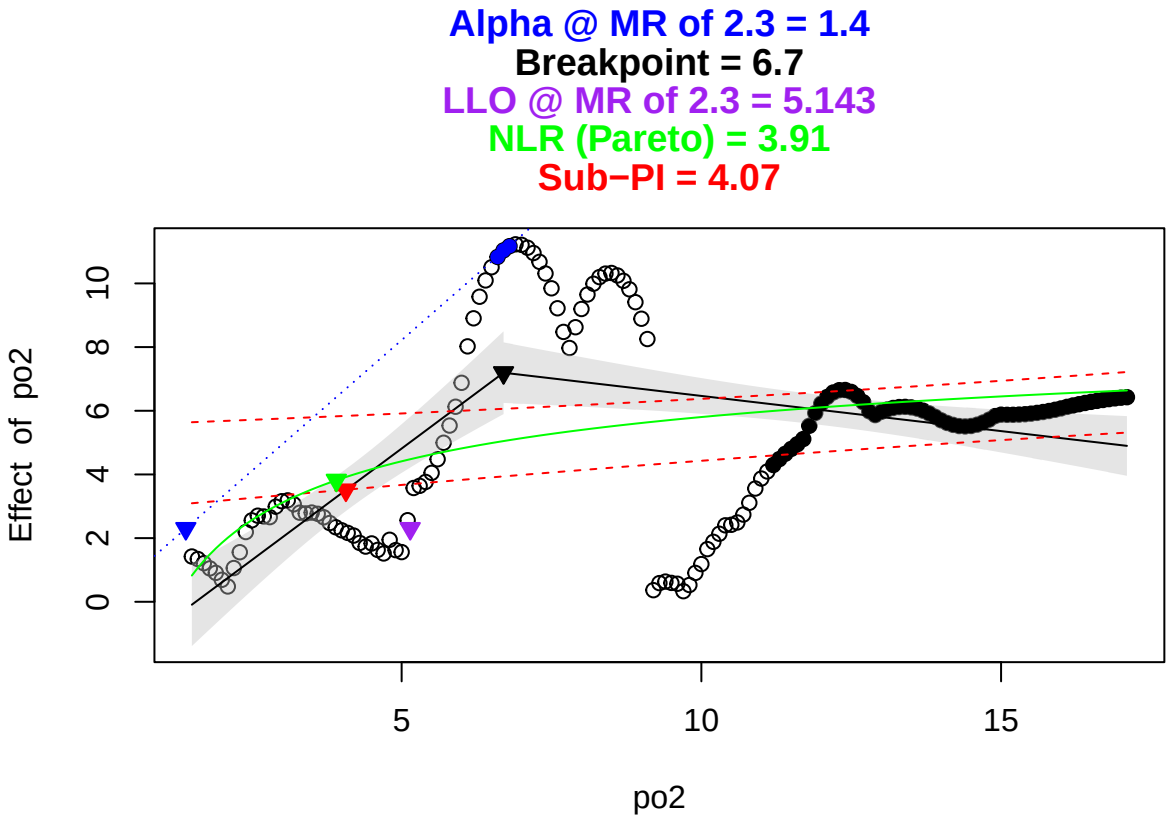

Alpha @ MR of 2.22 = 2.801
Breakpoint = 5.179
LLO @ MR of 2.22 = 4.792
NLR (Michaelis−Menten) = 2.554
Sub−PI = 4.75
Effect of po2
po2
0
1
2
3
4
2
4
6
8
10

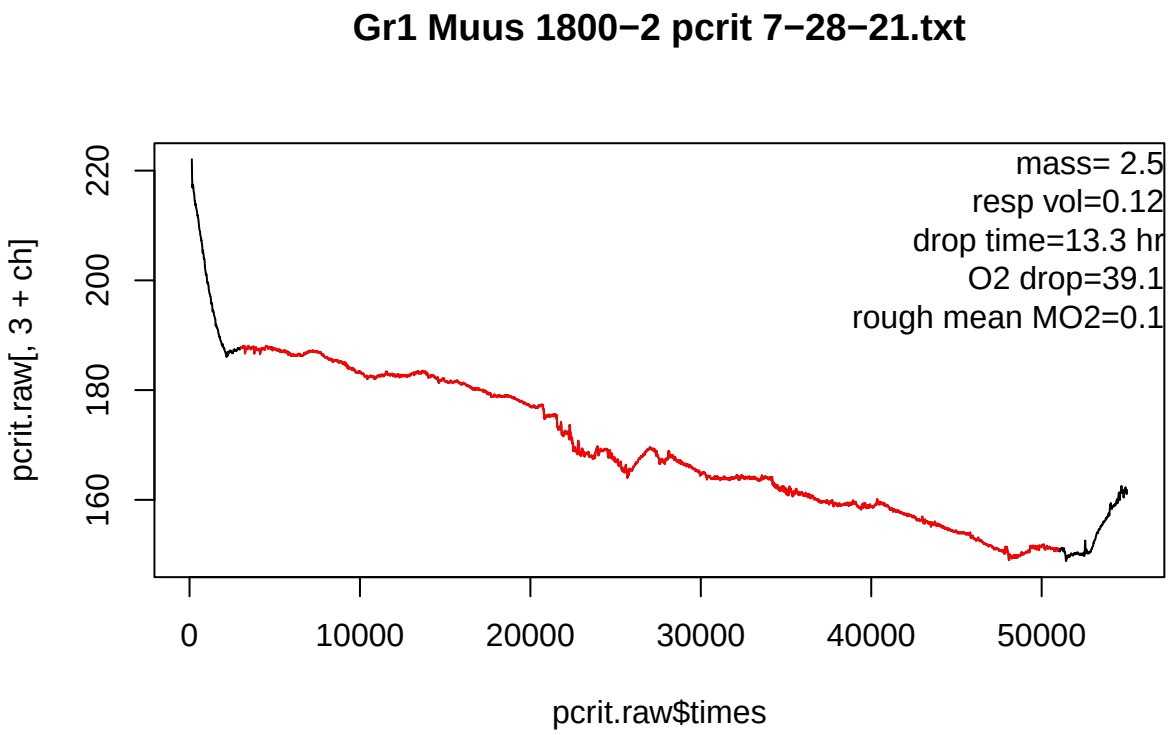

Gr1 Muus 1000−2 pcrit 7−27−21.txt
mass= 20.6
resp vol=1
drop time=1.5 hr
O2 drop=68.7
rough mean MO2=2.2
pcrit.raw[, 3 + ch]
170
190
210
230
0
1000
2000
3000
4000
5000
pcrit.raw$times

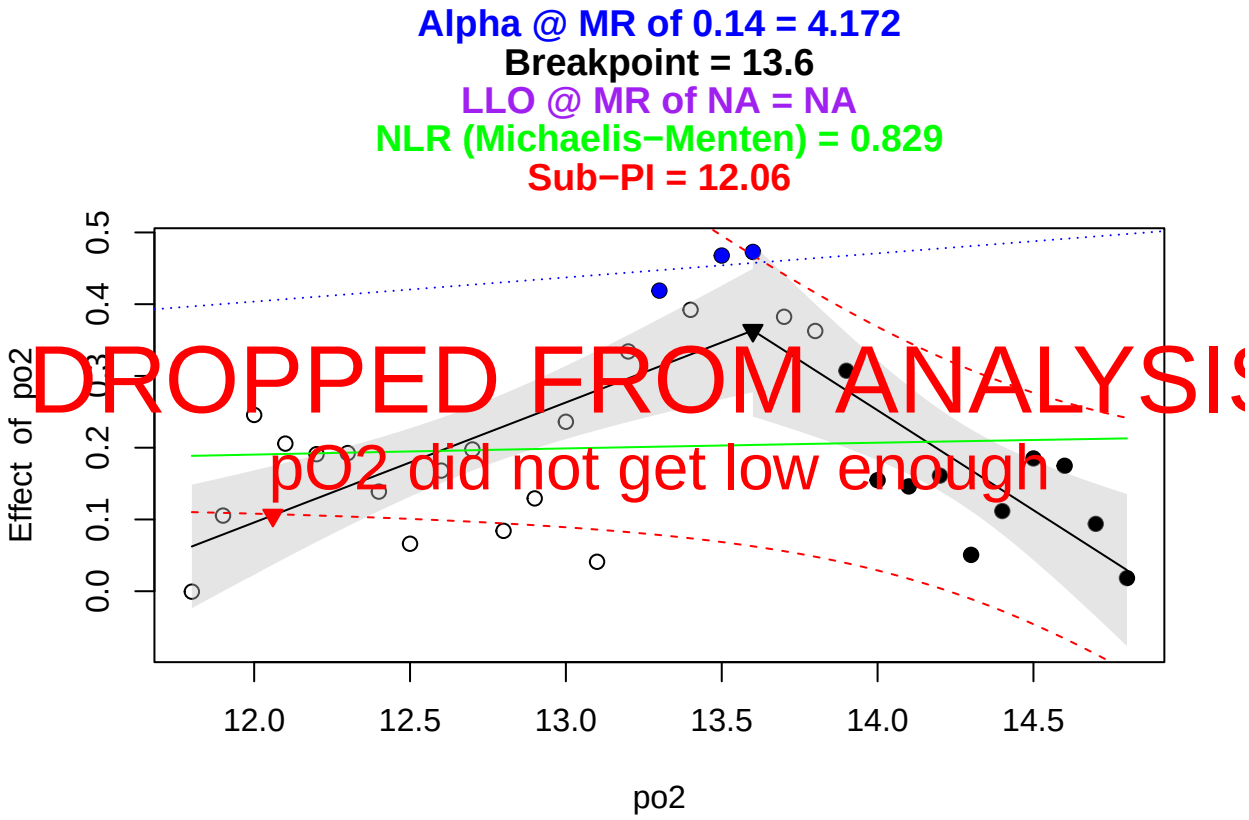
Alpha @ MR of 2.22 = 8.109
Breakpoint = 17.8
LLO @ MR of 2.22 = 13.235
NLR () = NA
Sub−PI = 18.7

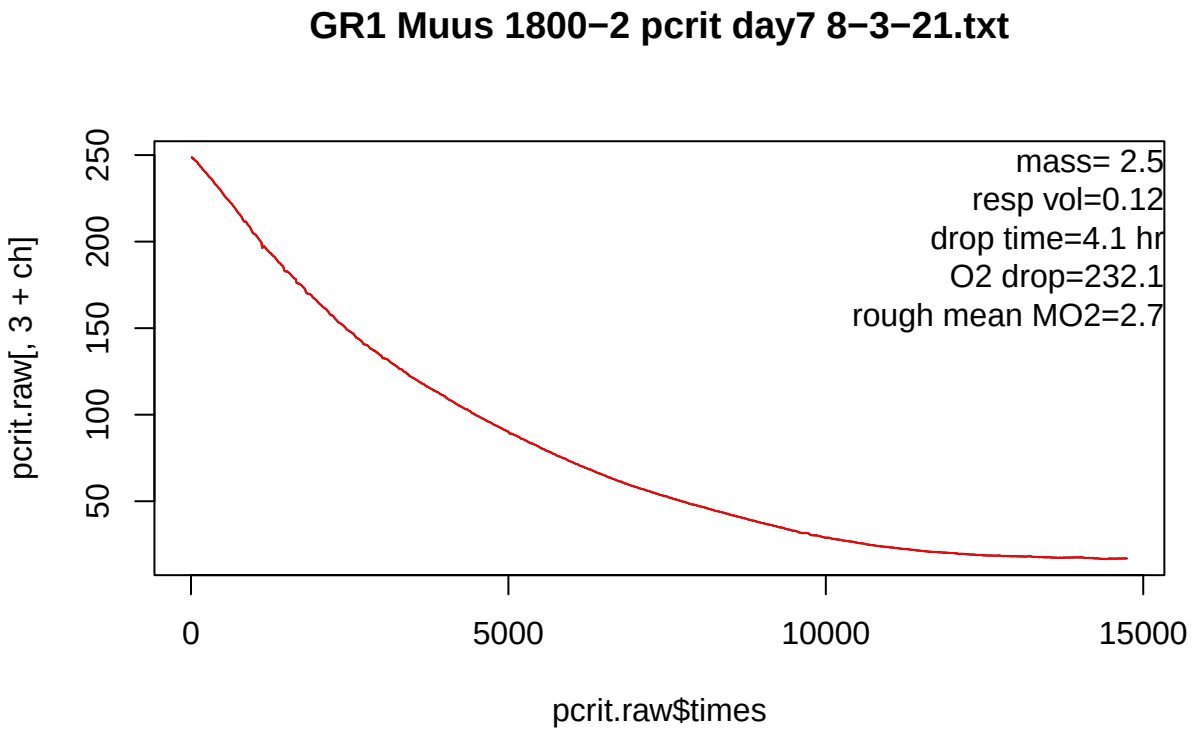


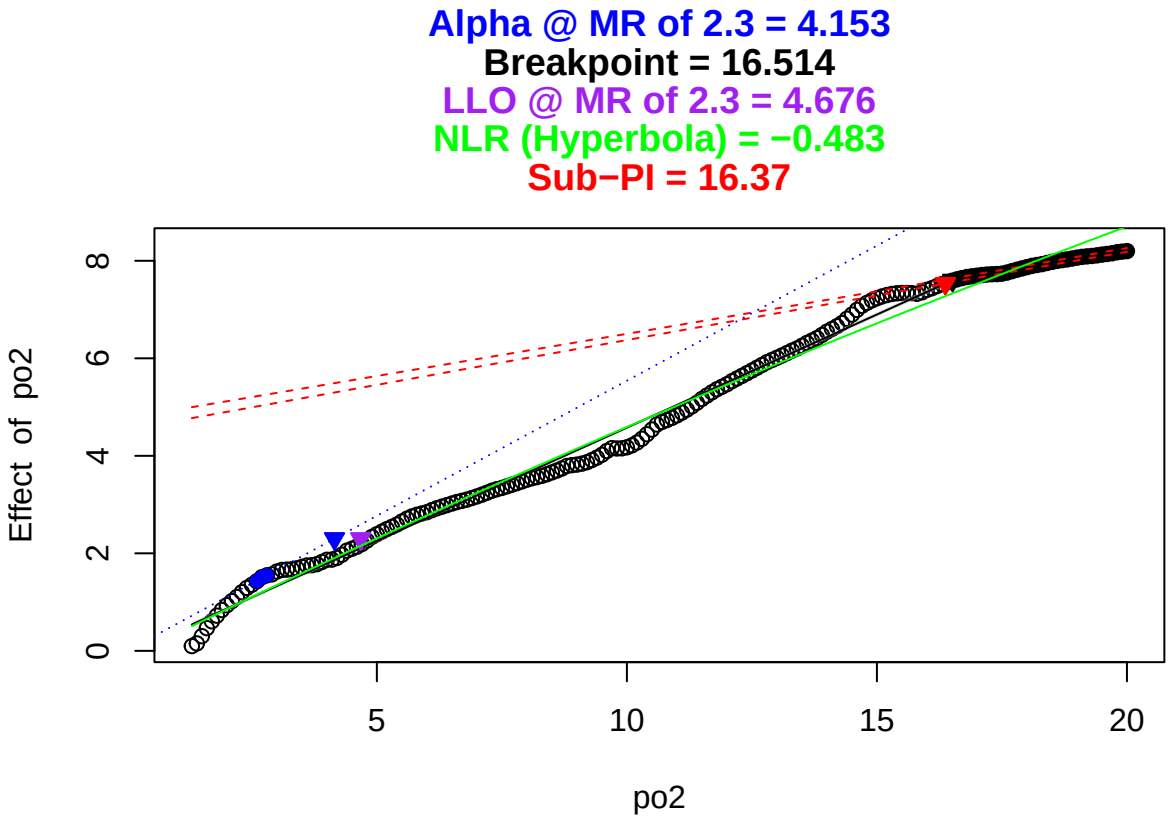

Gr1 Muus 1800−2 pcrit 25 ml jar 7−29−21 ch2 blank.txt
mass= 2.5
resp vol=0.025
drop time=0.7 hr
O2 drop=200.5
rough mean MO2=2.9
pcrit.raw[, 3 + ch]
50
150
250
0
1000
2000
3000
4000
5000
pcrit.raw$times

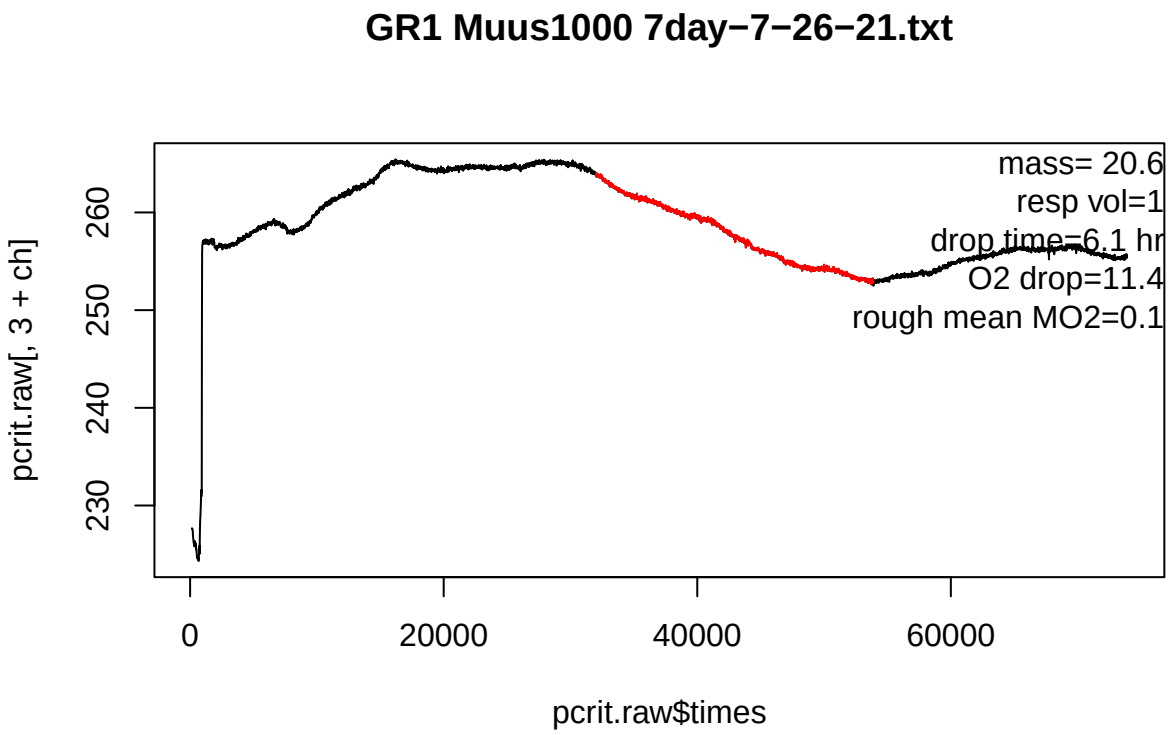

Alpha @ MR of 2.3 = 1.4
Breakpoint = 6.7
LLO @ MR of 2.3 = 5.143
NLR (Pareto) = 3.91
Sub−PI = 4.07
Effect of po2
0
2
4
6
8
10
5
10
15
po2


Gr1 Muus 1800−2 pcrit 7−28−21.txt
mass= 2.5
resp vol=0.12
drop time=13.3 hr
O2 drop=39.1
rough mean MO2=0.1
pcrit.raw[, 3 + ch]
160
180
200
220
0
10000
20000
30000
40000
50000
pcrit.raw$times

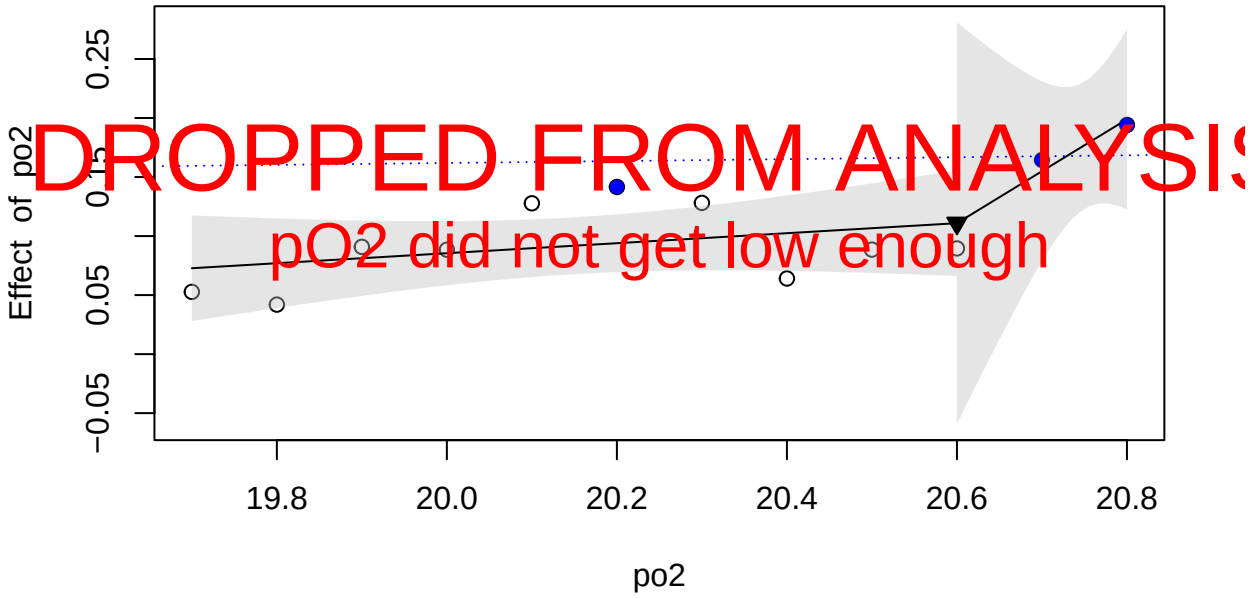

Alpha @ MR of 0.14 = 4.172
Breakpoint = 13.6
LLO @ MR of NA = NA
NLR (Michaelis−Menten) = 0.829
Sub−PI = 12.06
DROPPED FROM ANALYSIS
pO2 did not get low enough
Effect of po2
0.0
0.1
0.2
0.3
0.4
0.5
12.0
12.5
13.0
13.5
14.0
14.5
po2

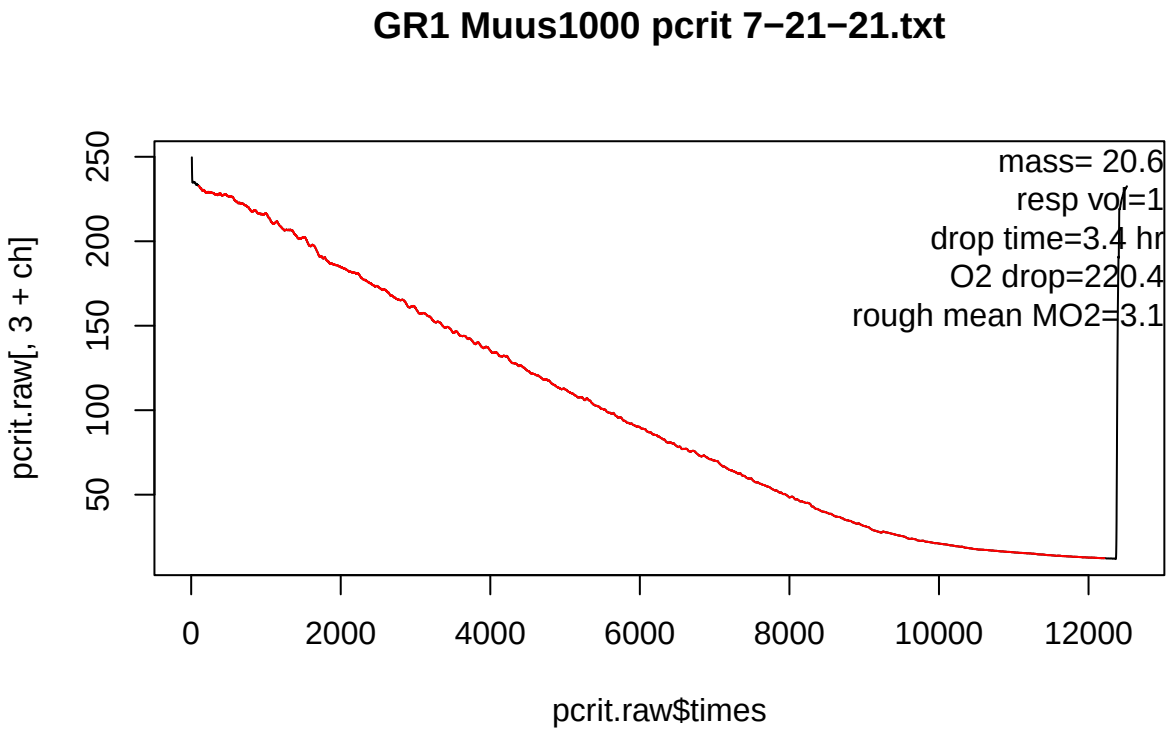

GR1 Muus 1800−2 pcrit day7 8−3−21.txt
mass= 2.5
resp vol=0.12
drop time=4.1 hr
O2 drop=232.1
rough mean MO2=2.7
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
pcrit.raw$times

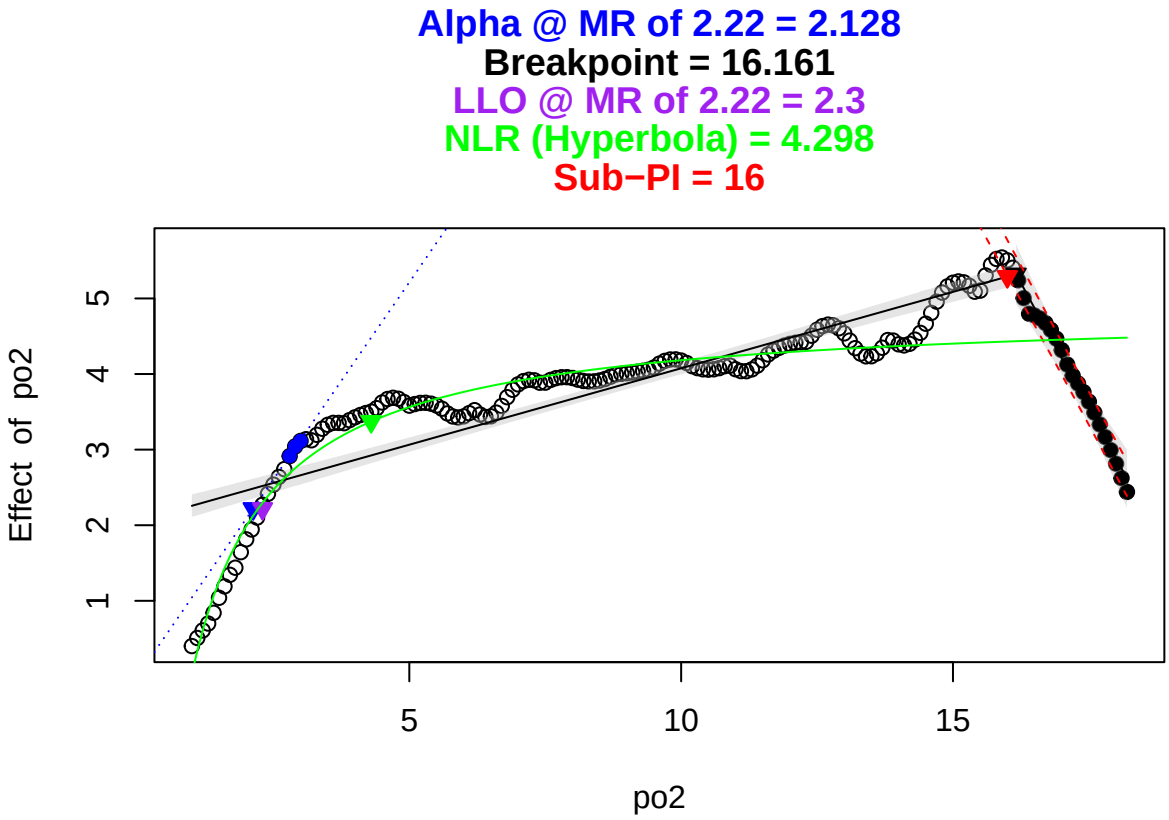

Alpha @ MR of 2.3 = 4.153
Breakpoint = 16.514
LLO @ MR of 2.3 = 4.676
NLR (Hyperbola) = −0.483
Sub−PI = 16.37
Effect of po2
0
2
4
6
8
5
10
15
20
po2

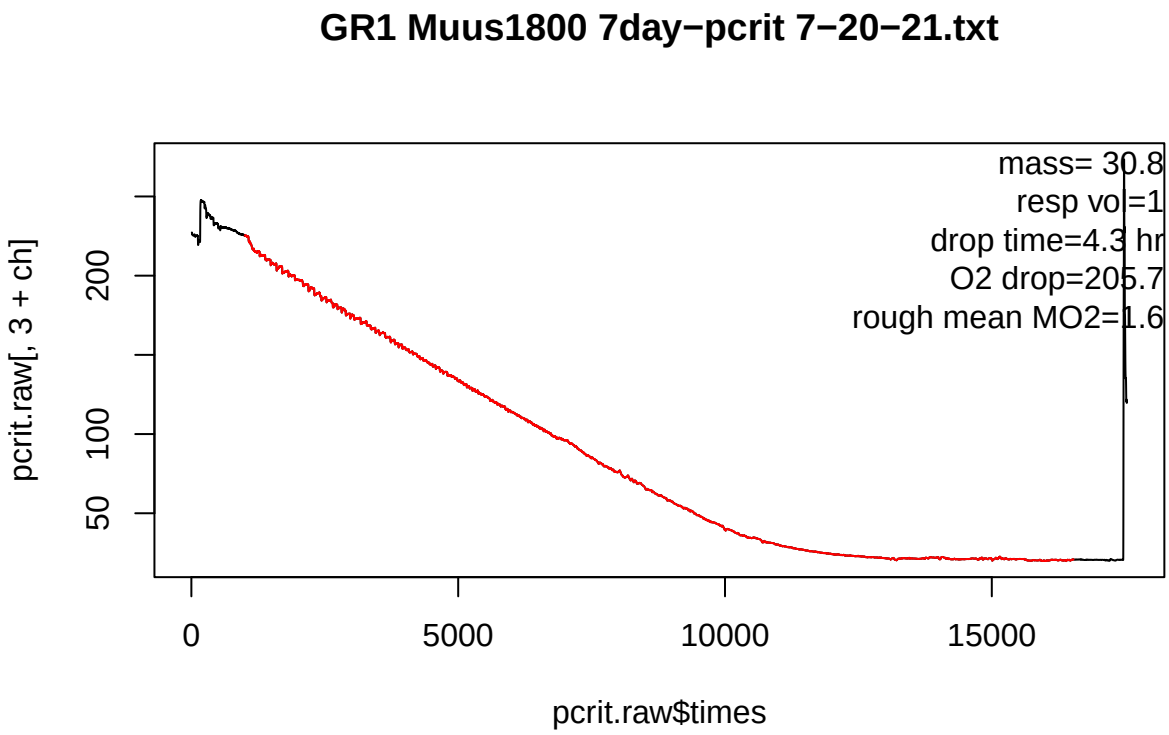

GR1 Muus1000 7day−7−26−21.txt
mass= 20.6
resp vol=1
drop time=6.1 hr
O2 drop=11.4
rough mean MO2=0.1
pcrit.raw[, 3 + ch]
230
240
250
260
0
20000
40000
60000
pcrit.raw$times

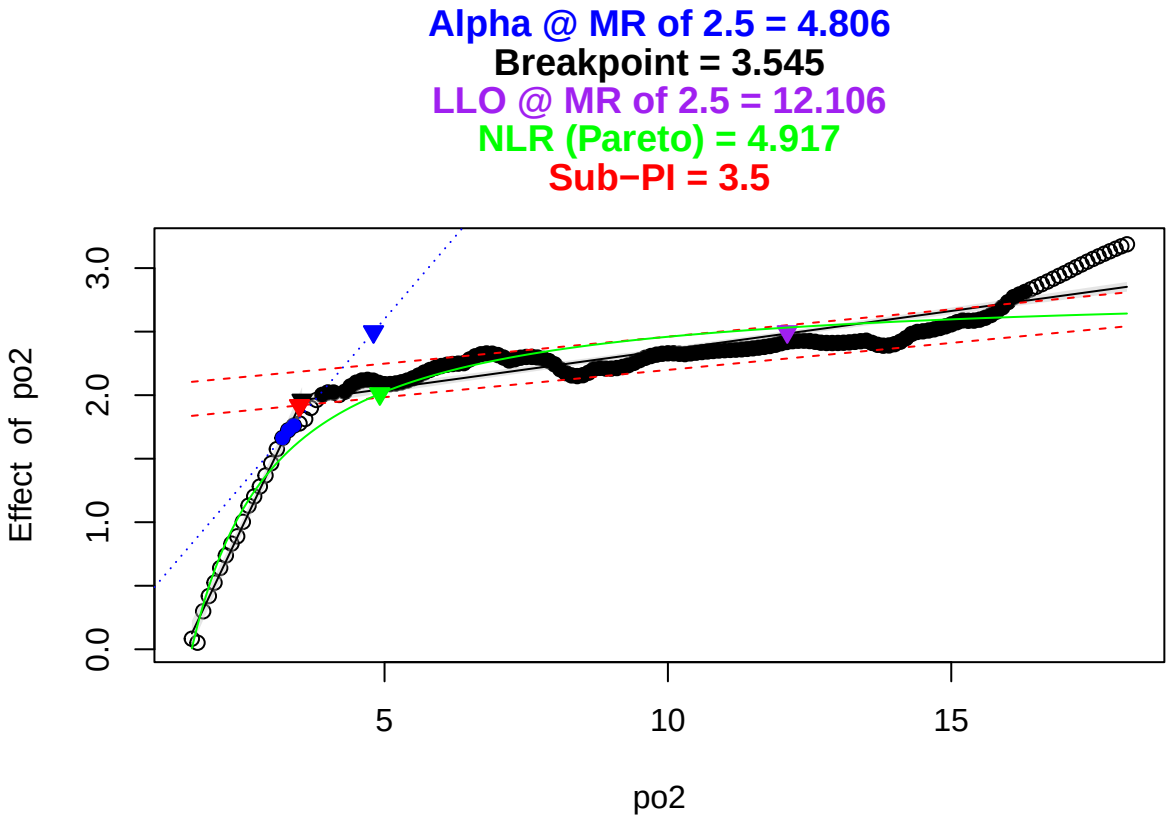
Alpha @ MR of 0.18 = 22.147
Breakpoint = 20.6
LLO @ MR of NA = NA
NLR () = NA
Sub−PI =

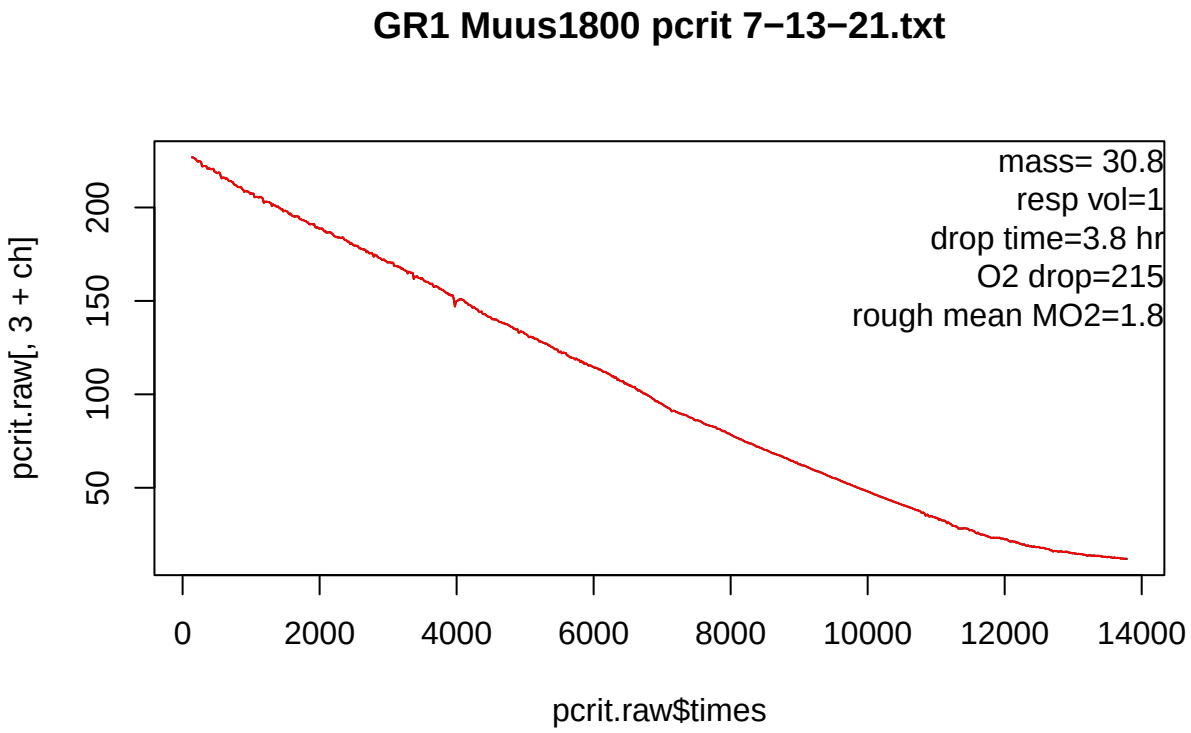


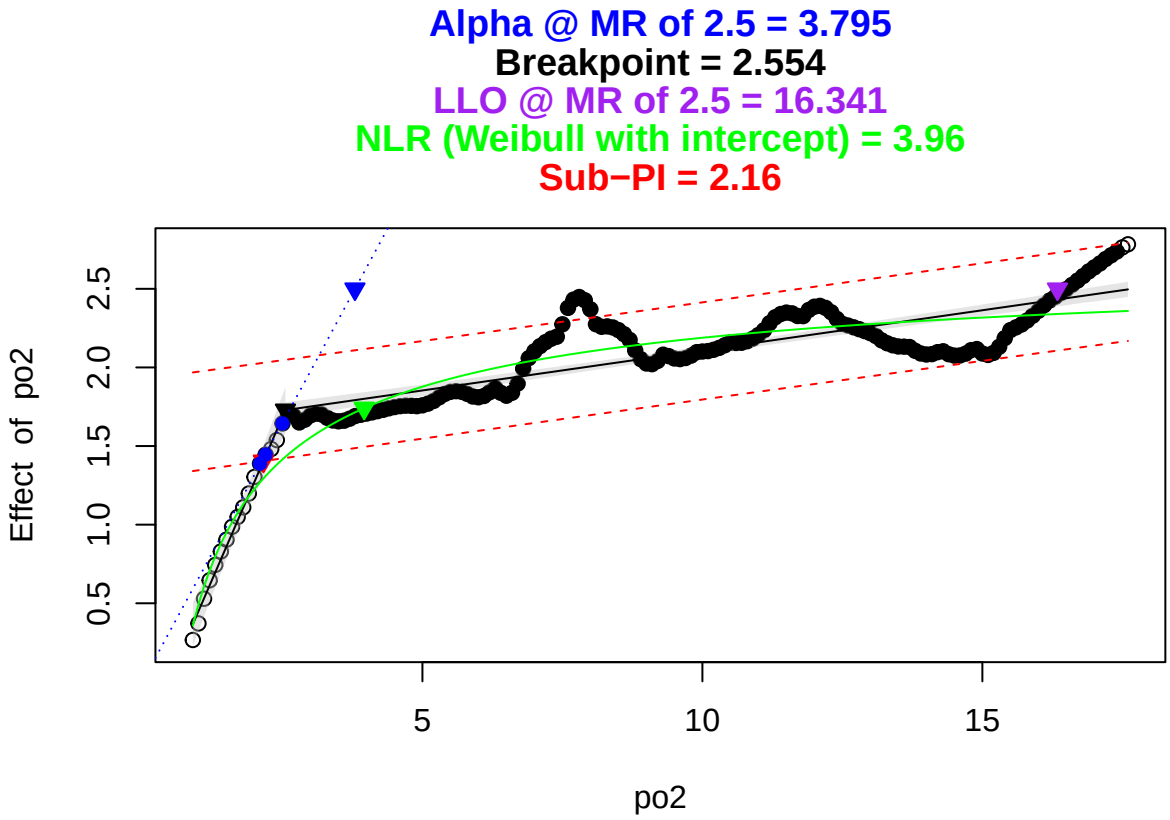

GR1 Muus1000 pcrit 7−21−21.txt
mass= 20.6
resp vol=1
drop time=3.4 hr
O2 drop=220.4
rough mean MO2=3.1
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
2000
4000
6000
8000
10000
12000
pcrit.raw$times

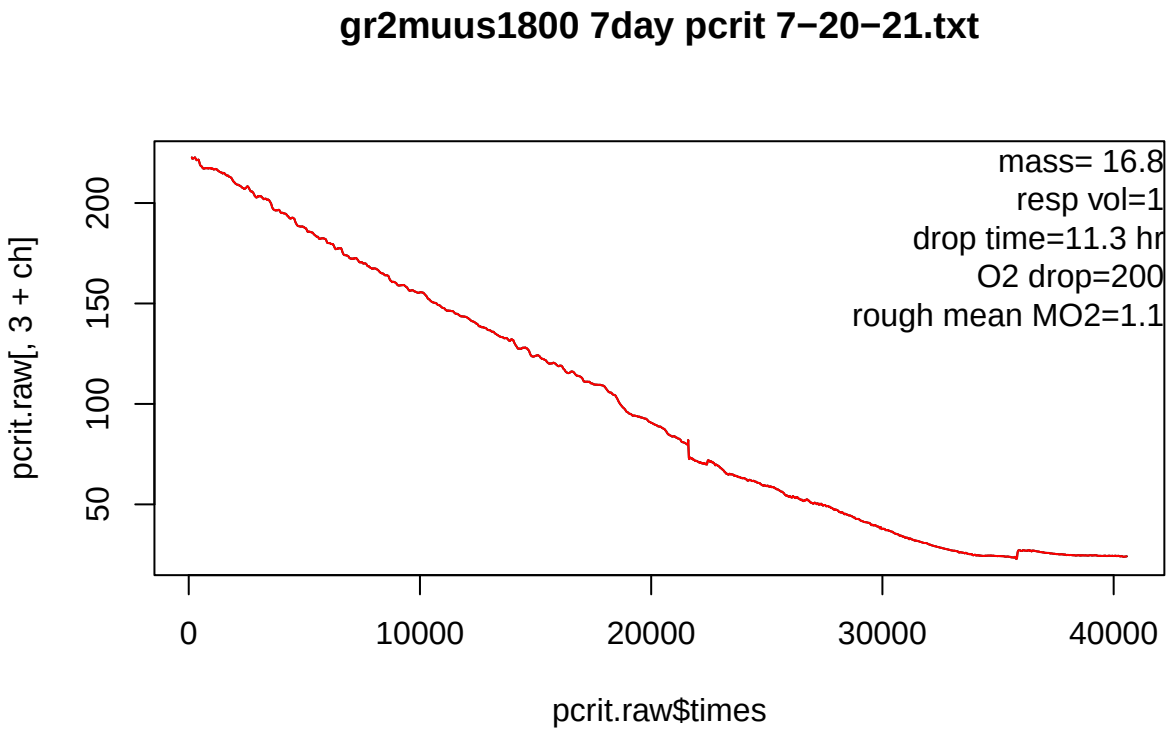

Alpha @ MR of 2.22 = 2.128
Breakpoint = 16.161
LLO @ MR of 2.22 = 2.3
NLR (Hyperbola) = 4.298
Sub−PI = 16
Effect of po2
1
2
3
4
5
5
10
15
po2

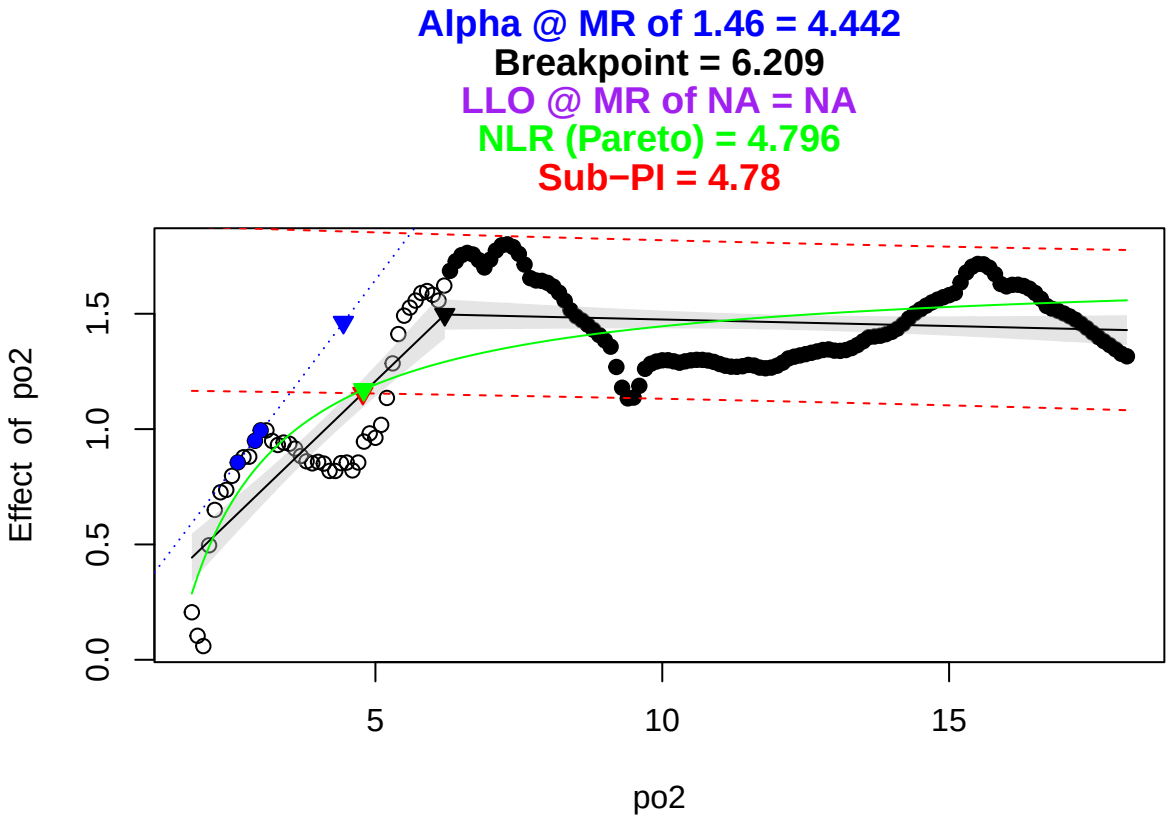

GR1 Muus1800 7day−pcrit 7−20−21.txt
mass= 30.8
resp vol=1
drop time=4.3 hr
O2 drop=205.7
rough mean MO2=1.6
pcrit.raw[, 3 + ch]
50
100
200
0
5000
10000
15000
pcrit.raw$times

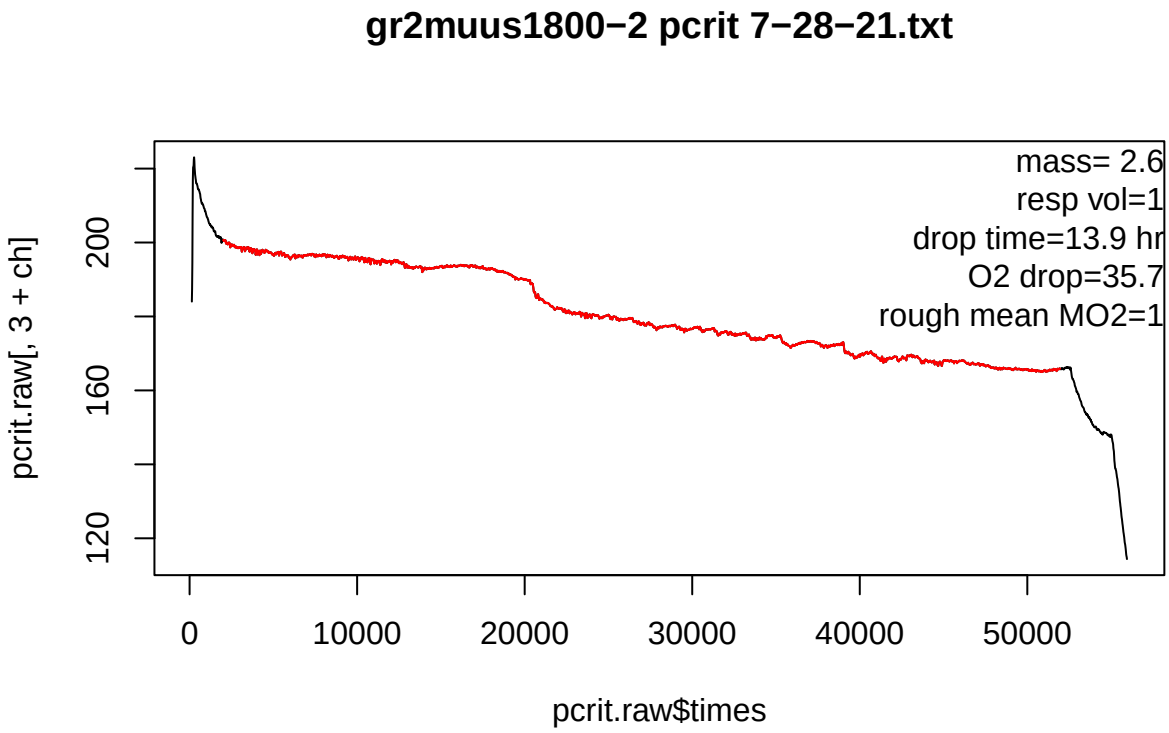

Alpha @ MR of 2.5 = 4.806
Breakpoint = 3.545
LLO @ MR of 2.5 = 12.106
NLR (Pareto) = 4.917
Sub−PI = 3.5
Effect of po2
0.0
1.0
2.0
3.0
5
10
15
po2

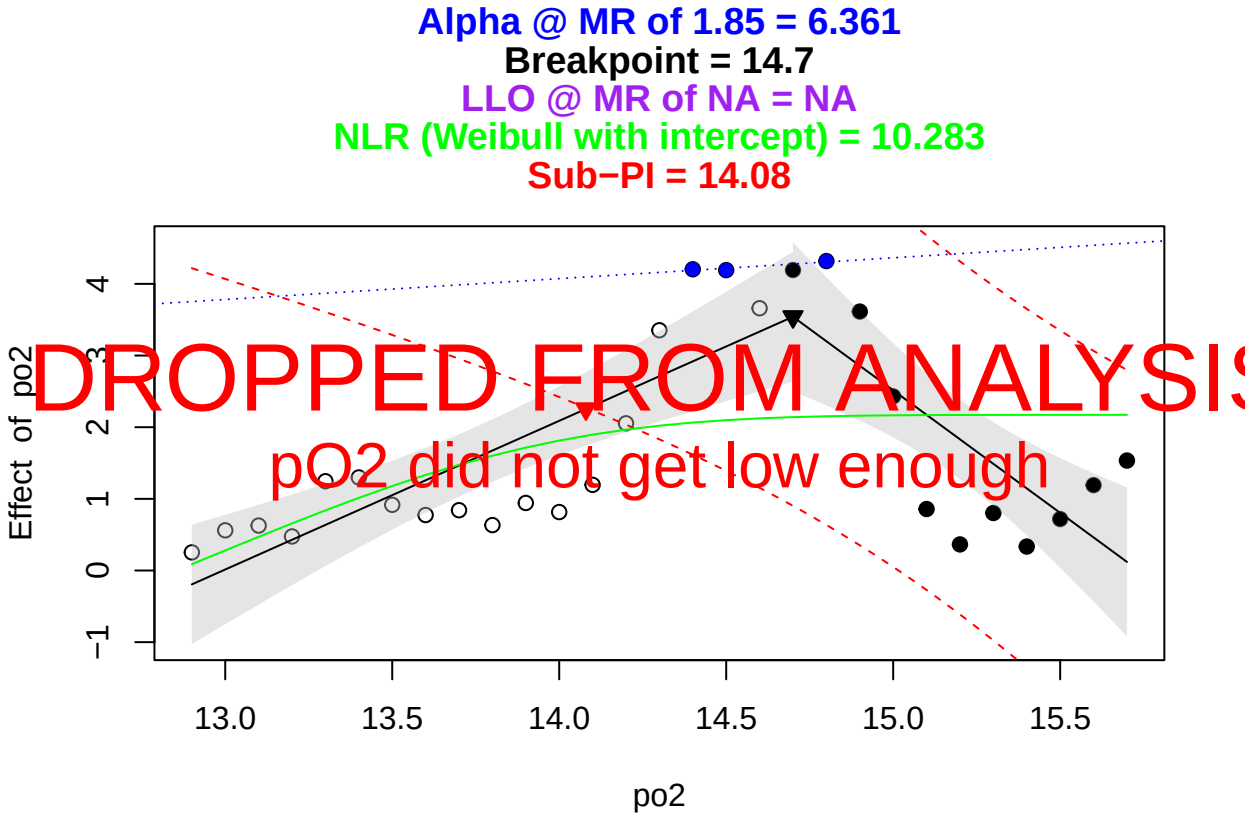

GR1 Muus1800 pcrit 7−13−21.txt
mass= 30.8
resp vol=1
drop time=3.8 hr
O2 drop=215
rough mean MO2=1.8
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
10000
12000
14000
pcrit.raw$times

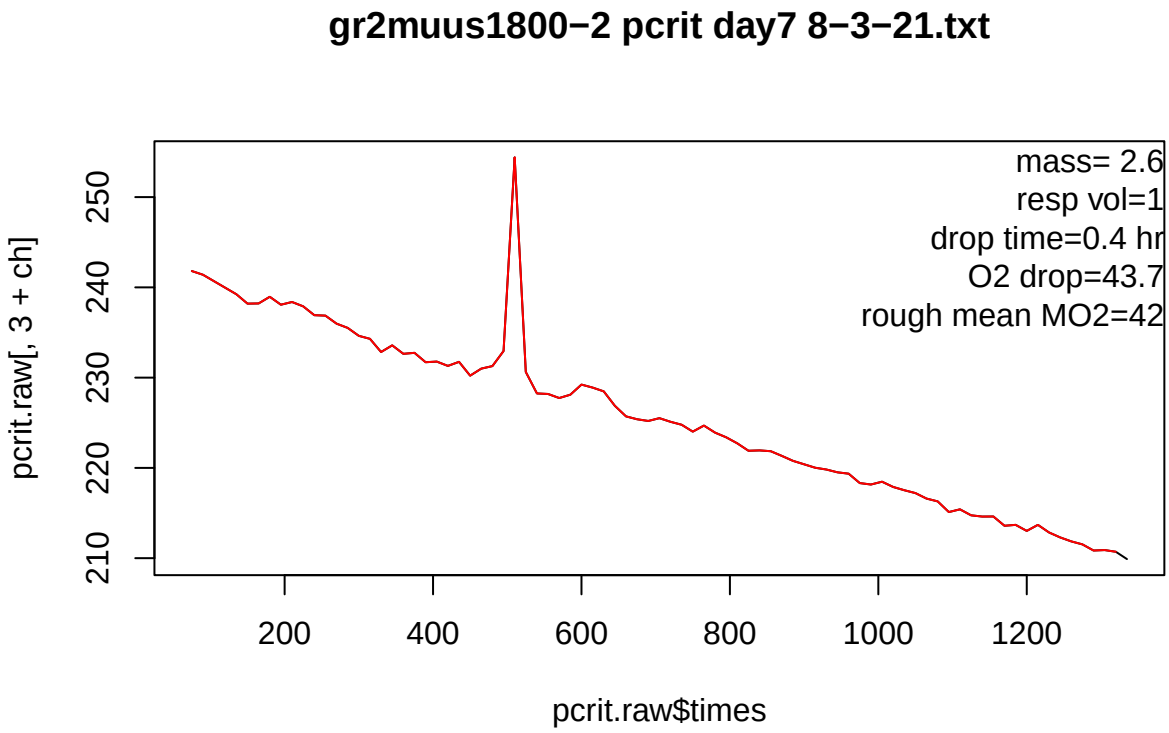

Alpha @ MR of 2.5 = 3.795
Breakpoint = 2.554
LLO @ MR of 2.5 = 16.341
NLR (Weibull with intercept) = 3.96
Sub−PI = 2.16
Effect of po2
0.5
1.0
1.5
2.0
2.5
5
10
15
po2


gr2muus1800 7day pcrit 7−20−21.txt
mass= 16.8
resp vol=1
drop time=11.3 hr
O2 drop=200
rough mean MO2=1.1
pcrit.raw[, 3 + ch]
50
100
150
200
0
10000
20000
30000
40000
pcrit.raw$times

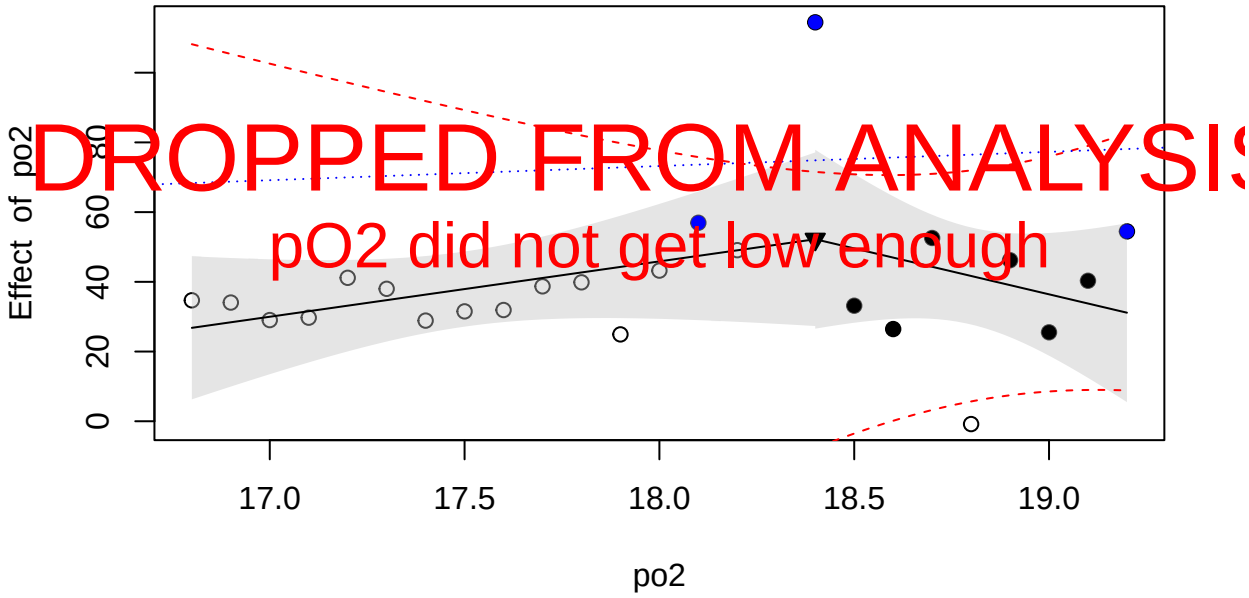

Alpha @ MR of 1.46 = 4.442
Breakpoint = 6.209
LLO @ MR of NA = NA
NLR (Pareto) = 4.796
Sub−PI = 4.78
Effect of po2
0.0
0.5
1.0
1.5
5
10
15
po2

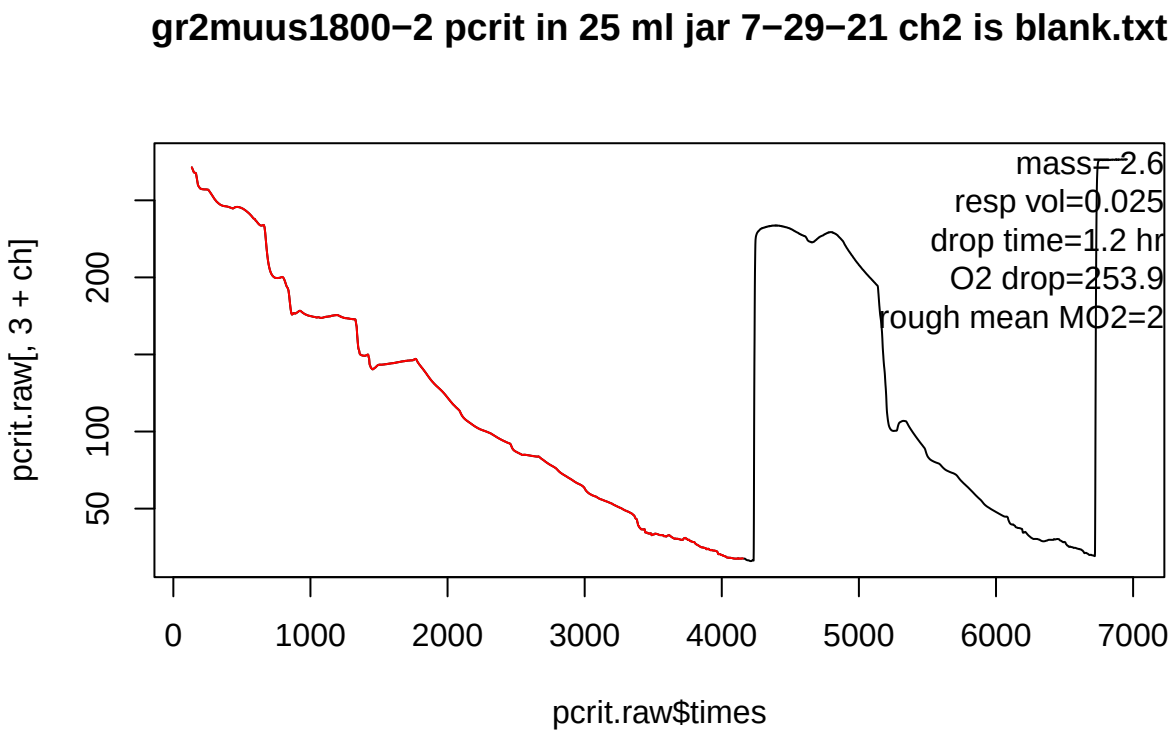

gr2muus1800−2 pcrit 7−28−21.txt
mass= 2.6
resp vol=1
drop time=13.9 hr
O2 drop=35.7
rough mean MO2=1
pcrit.raw[, 3 + ch]
120
160
200
0
10000
20000
30000
40000
50000
pcrit.raw$times

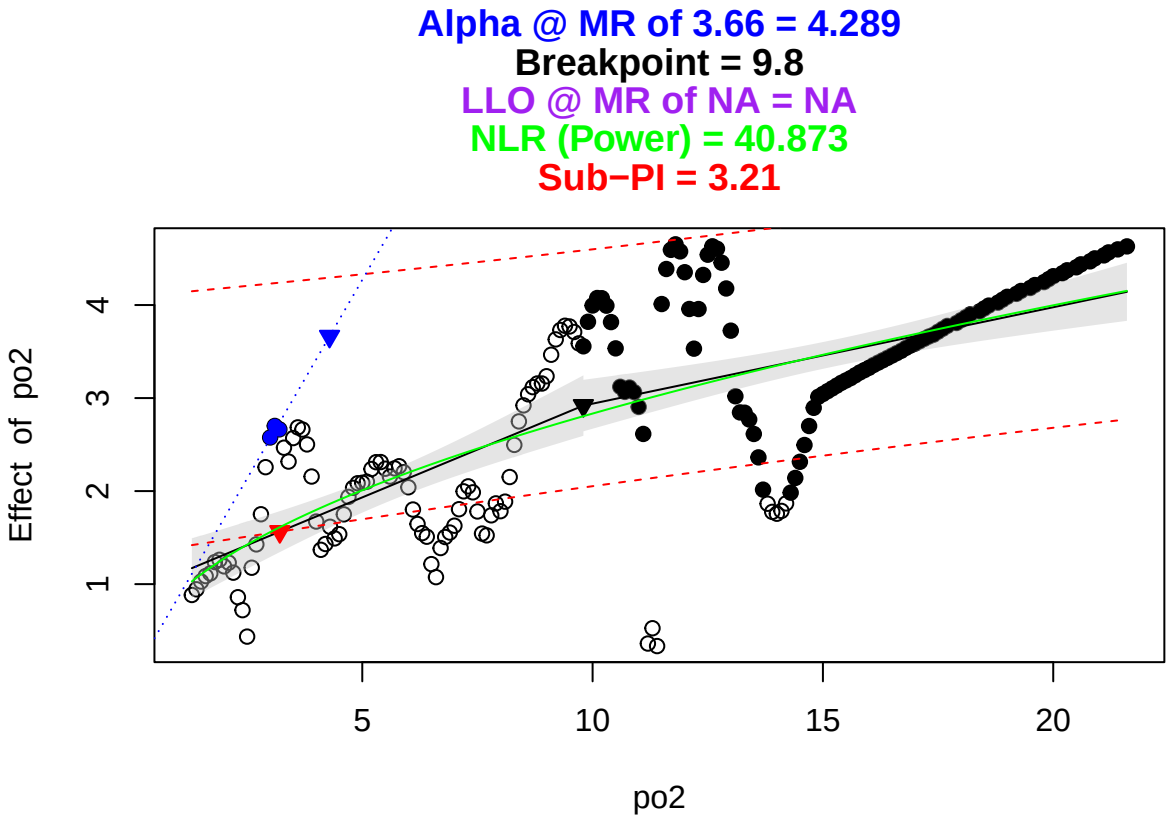

Alpha @ MR of 1.85 = 6.361
Breakpoint = 14.7
LLO @ MR of NA = NA
NLR (Weibull with intercept) = 10.283
Sub−PI = 14.08
DROPPED FROM ANALYSIS
pO2 did not get low enough
Effect of po2
−1
0
1
2
3
4
13.0
13.5
14.0
14.5
15.0
15.5
po2

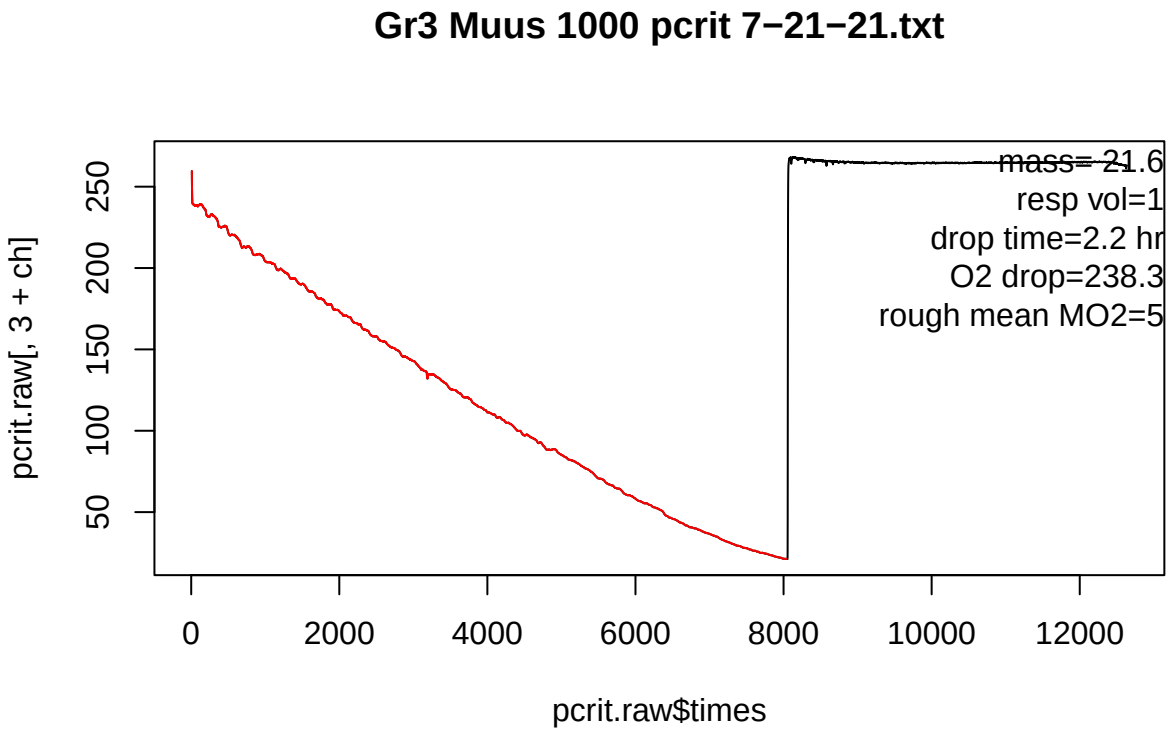

gr2muus1800−2 pcrit day7 8−3−21.txt
mass= 2.6
resp vol=1
drop time=0.4 hr
O2 drop=43.7
rough mean MO2=42
pcrit.raw[, 3 + ch]
210
220
230
240
250
200
400
600
800
1000
1200
pcrit.raw$times

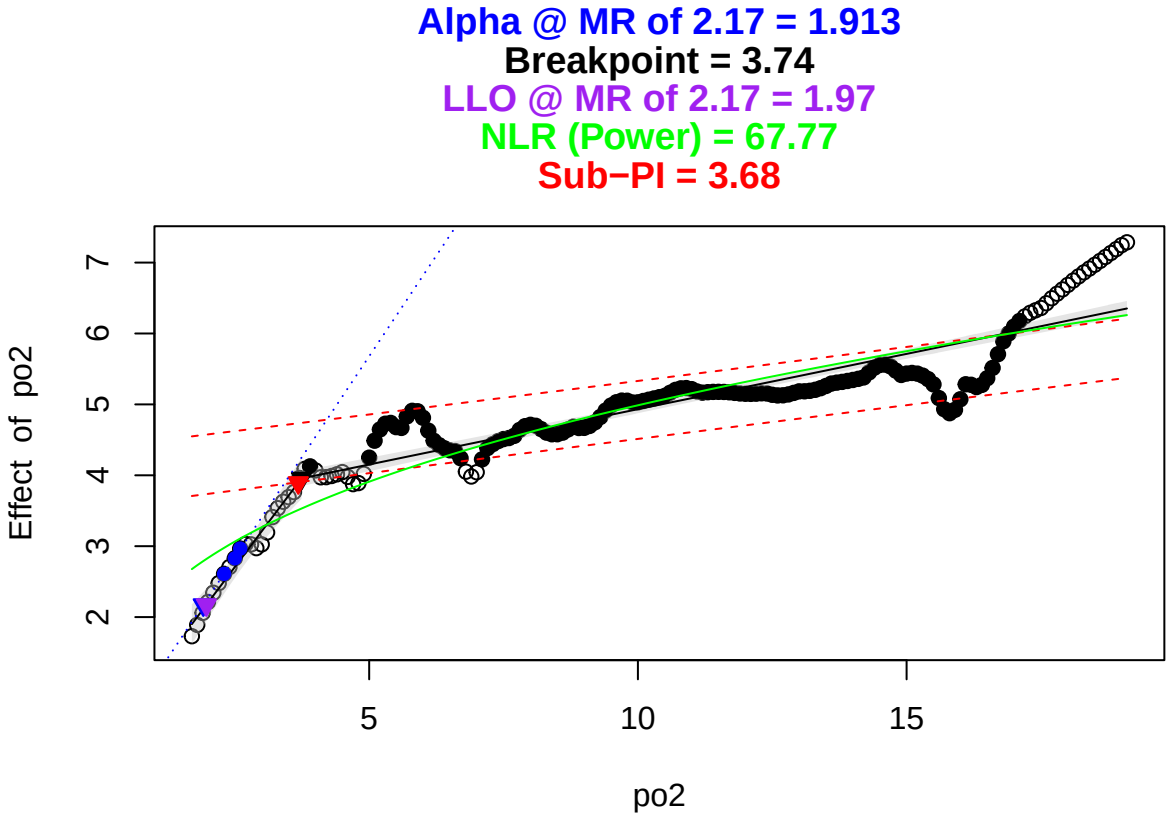
Alpha @ MR of 22.09 = 5.43
Breakpoint = 18.4
LLO @ MR of 22.09 = NA
NLR () = NA
Sub−PI =

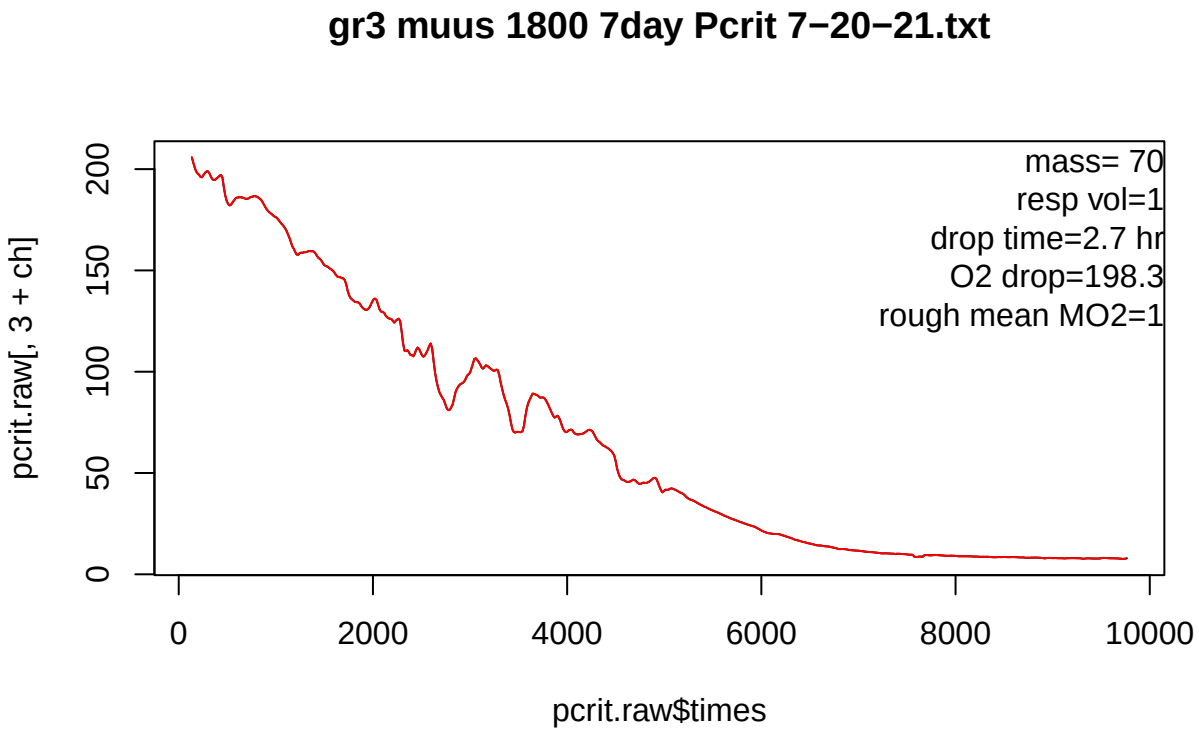


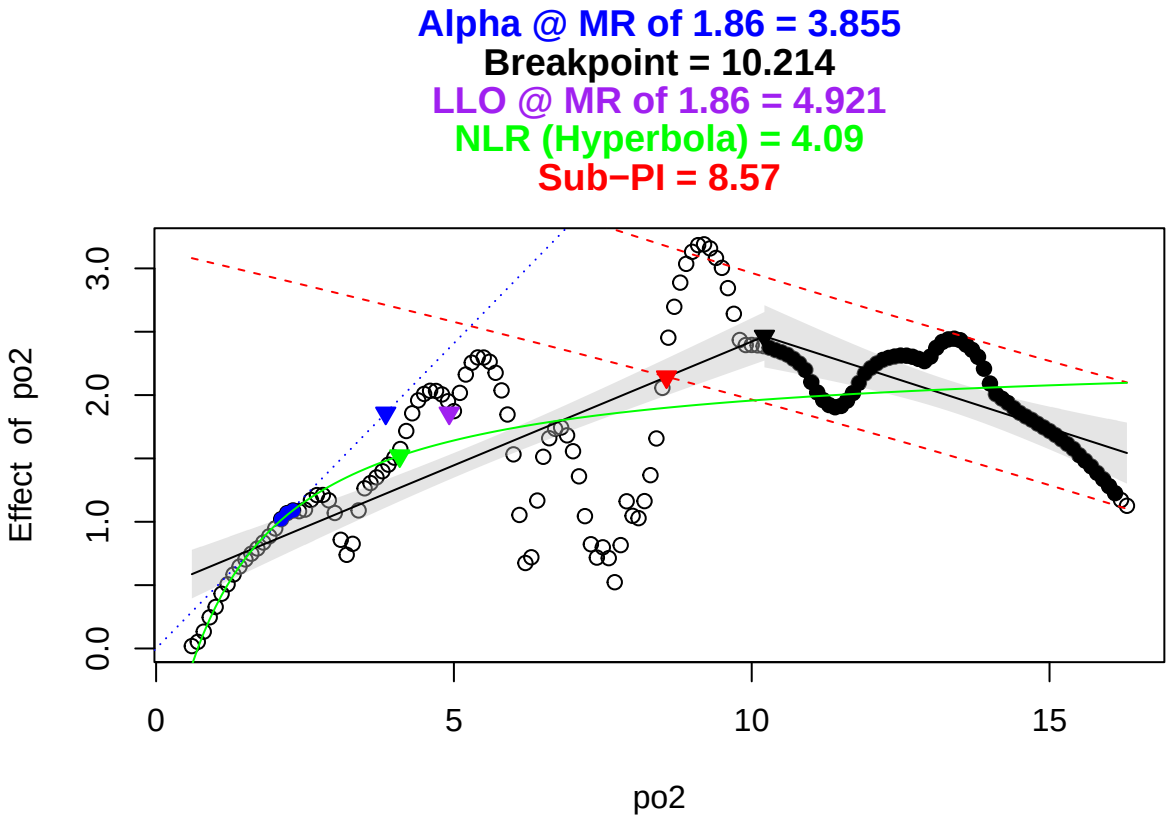

gr2muus1800−2 pcrit in 25 ml jar 7−29−21 ch2 is blank.txt
mass= 2.6
resp vol=0.025
drop time=1.2 hr
O2 drop=253.9
rough mean MO2=2
pcrit.raw[, 3 + ch]
50
100
200
0
1000
2000
3000
4000
5000
6000
7000
pcrit.raw$times

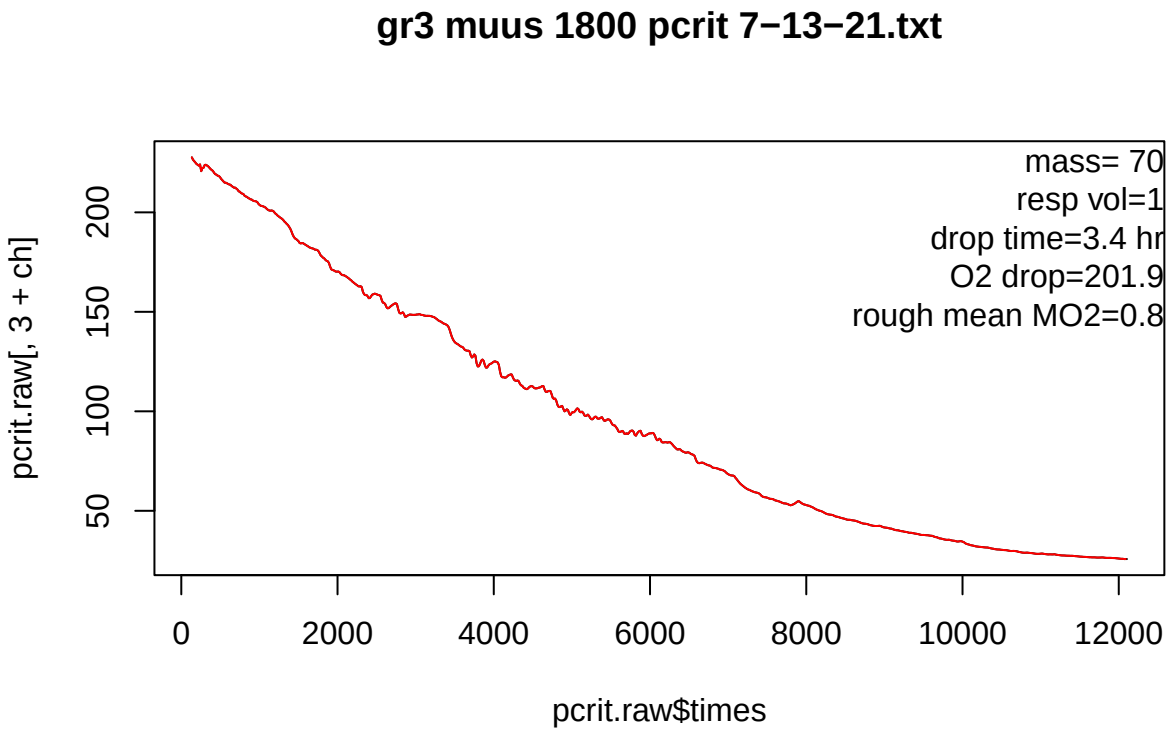

Alpha @ MR of 3.66 = 4.289
Breakpoint = 9.8
LLO @ MR of NA = NA
NLR (Power) = 40.873
Sub−PI = 3.21
Effect of po2
1
2
3
4
5
10
15
20
po2

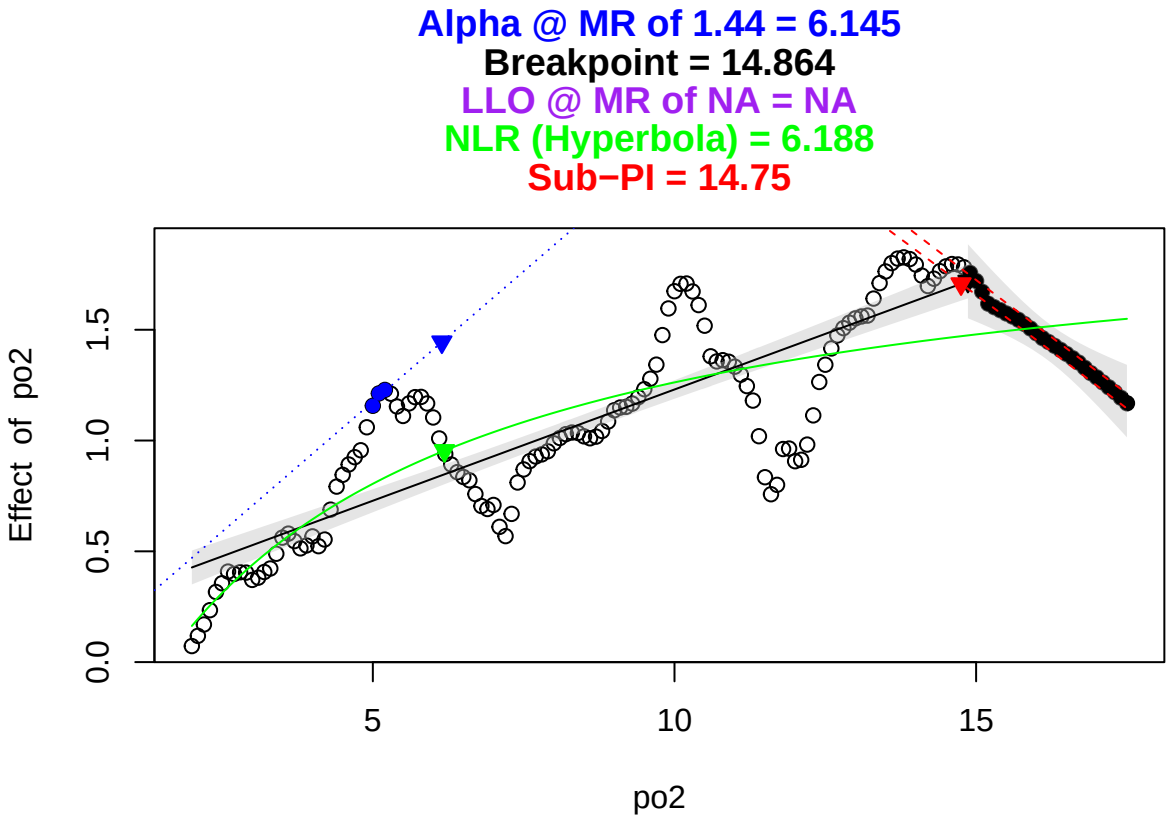

Gr3 Muus 1000 pcrit 7−21−21.txt
mass= 21.6
resp vol=1
drop time=2.2 hr
O2 drop=238.3
rough mean MO2=5
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
2000
4000
6000
8000
10000
12000
pcrit.raw$times

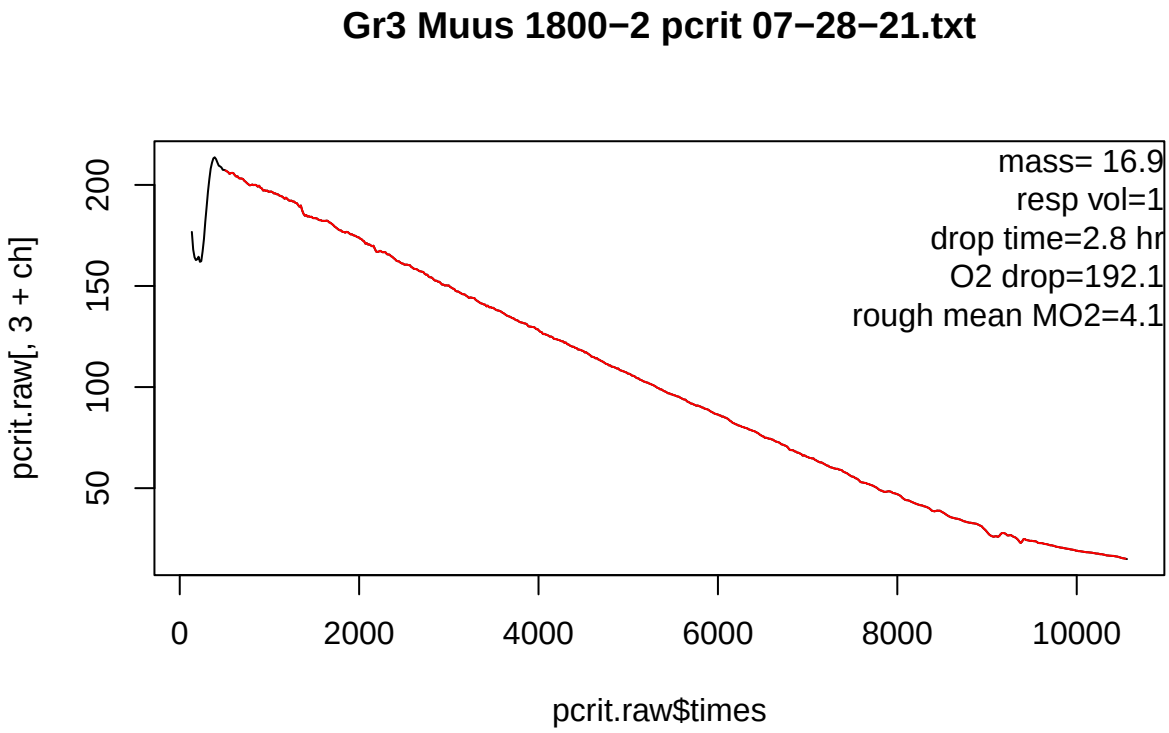

Alpha @ MR of 2.17 = 1.913
Breakpoint = 3.74
LLO @ MR of 2.17 = 1.97
NLR (Power) = 67.77
Sub−PI = 3.68
Effect of po2
2
3
4
5
6
7
5
10
15
po2

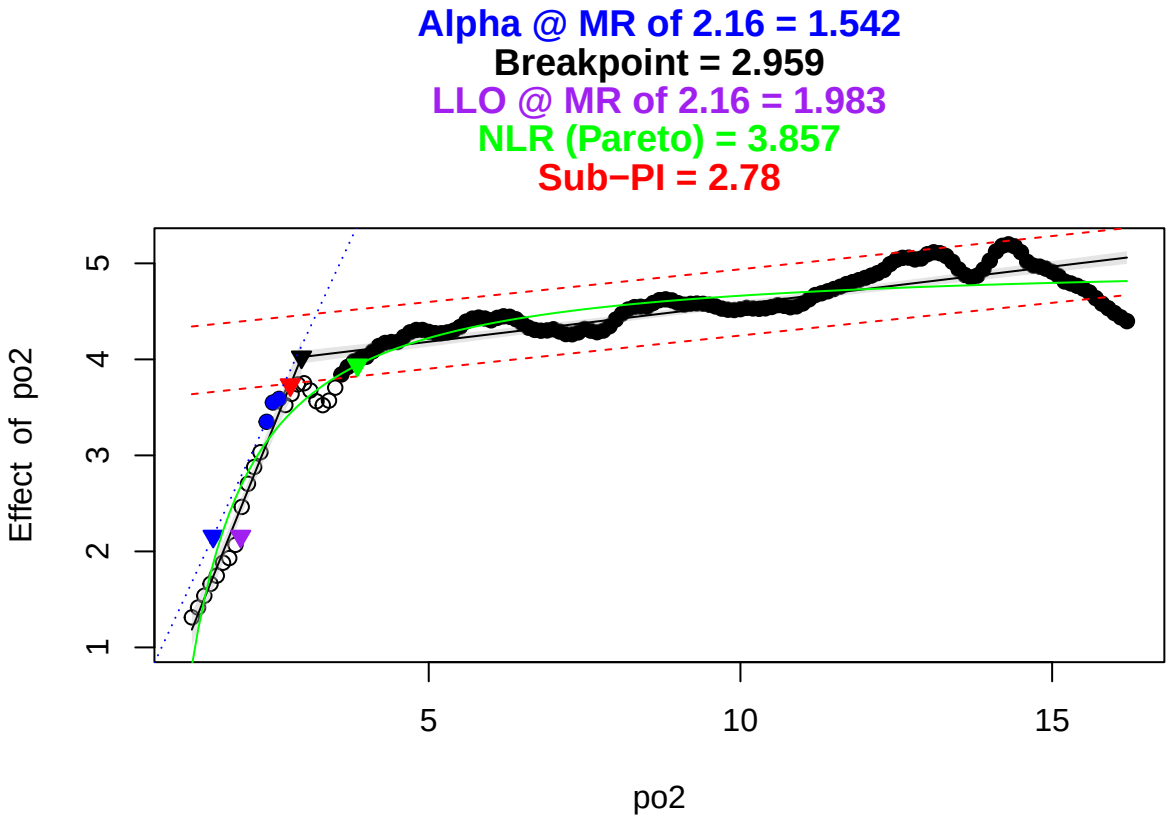

gr3 muus 1800 7day Pcrit 7−20−21.txt
mass= 70
resp vol=1
drop time=2.7 hr
O2 drop=198.3
rough mean MO2=1
pcrit.raw[, 3 + ch]
0
50
100
150
200
0
2000
4000
6000
8000
10000
pcrit.raw$times

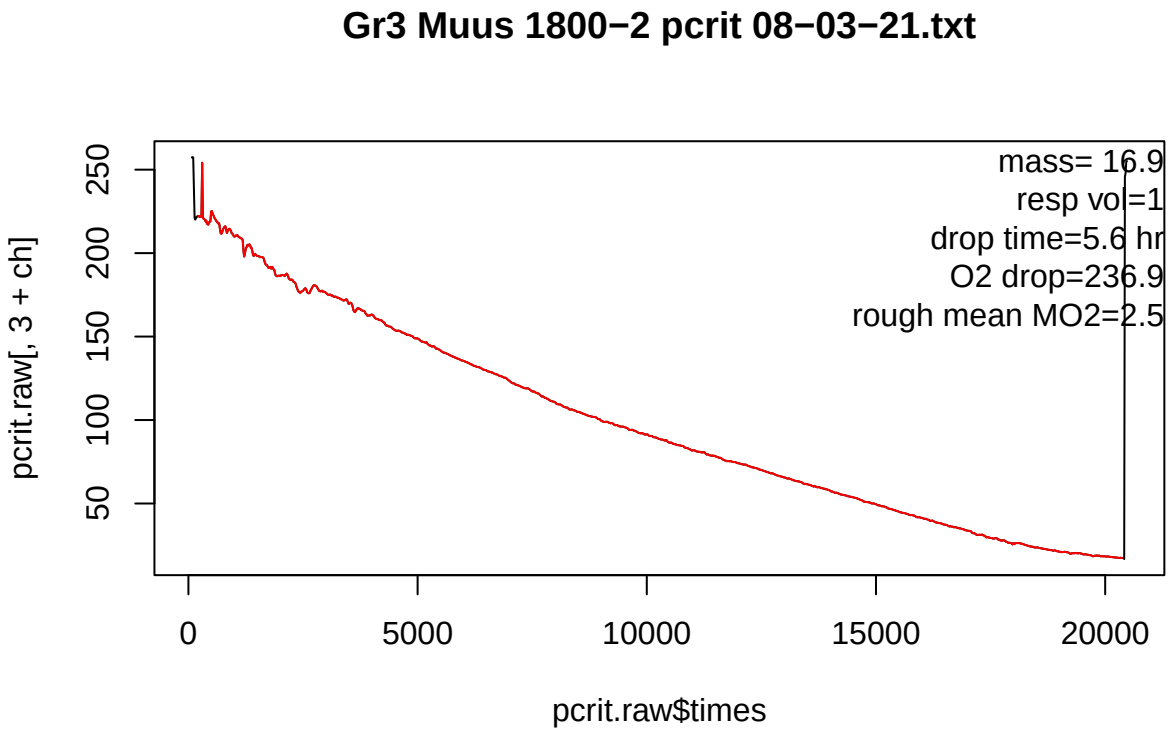

Alpha @ MR of 1.86 = 3.855
Breakpoint = 10.214
LLO @ MR of 1.86 = 4.921
NLR (Hyperbola) = 4.09
Sub−PI = 8.57
Effect of po2
0.0
1.0
2.0
3.0
0
5
10
15
po2

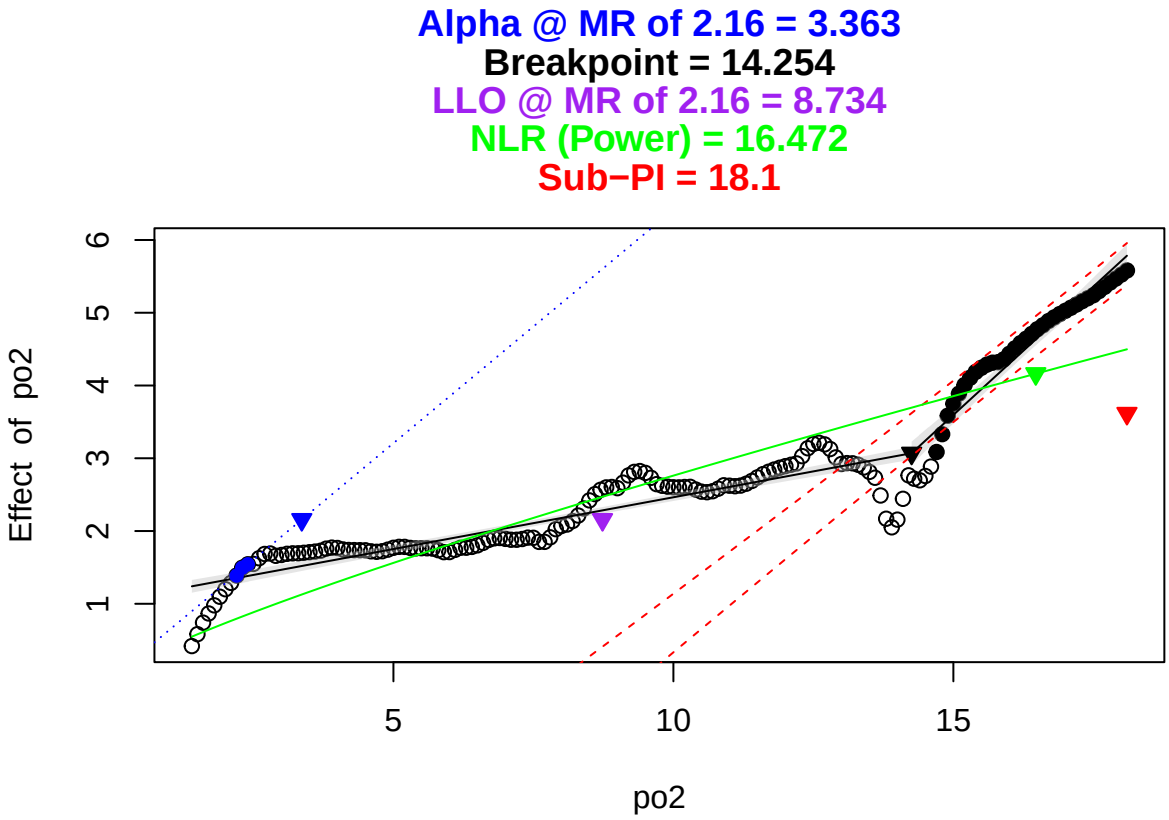

gr3 muus 1800 pcrit 7−13−21.txt
mass= 70
resp vol=1
drop time=3.4 hr
O2 drop=201.9
rough mean MO2=0.8
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
10000
12000
pcrit.raw$times

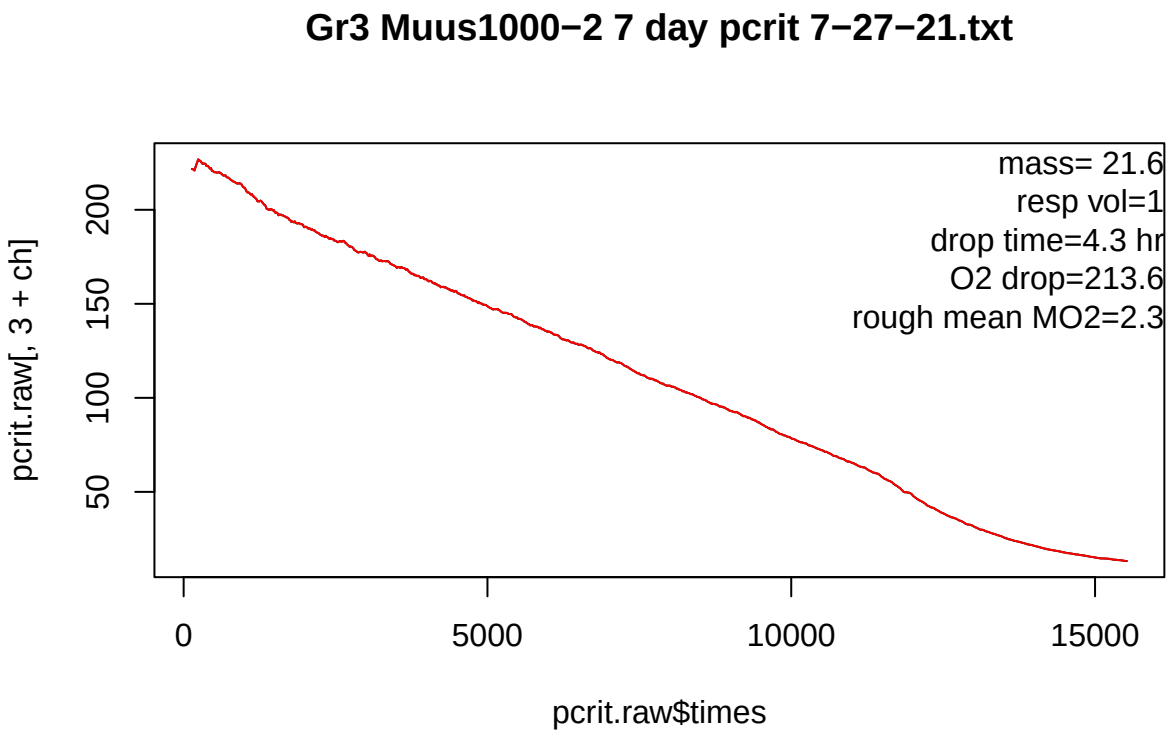

Alpha @ MR of 1.44 = 6.145
Breakpoint = 14.864
LLO @ MR of NA = NA
NLR (Hyperbola) = 6.188
Sub−PI = 14.75
Effect of po2
0.0
0.5
1.0
1.5
5
10
15
po2

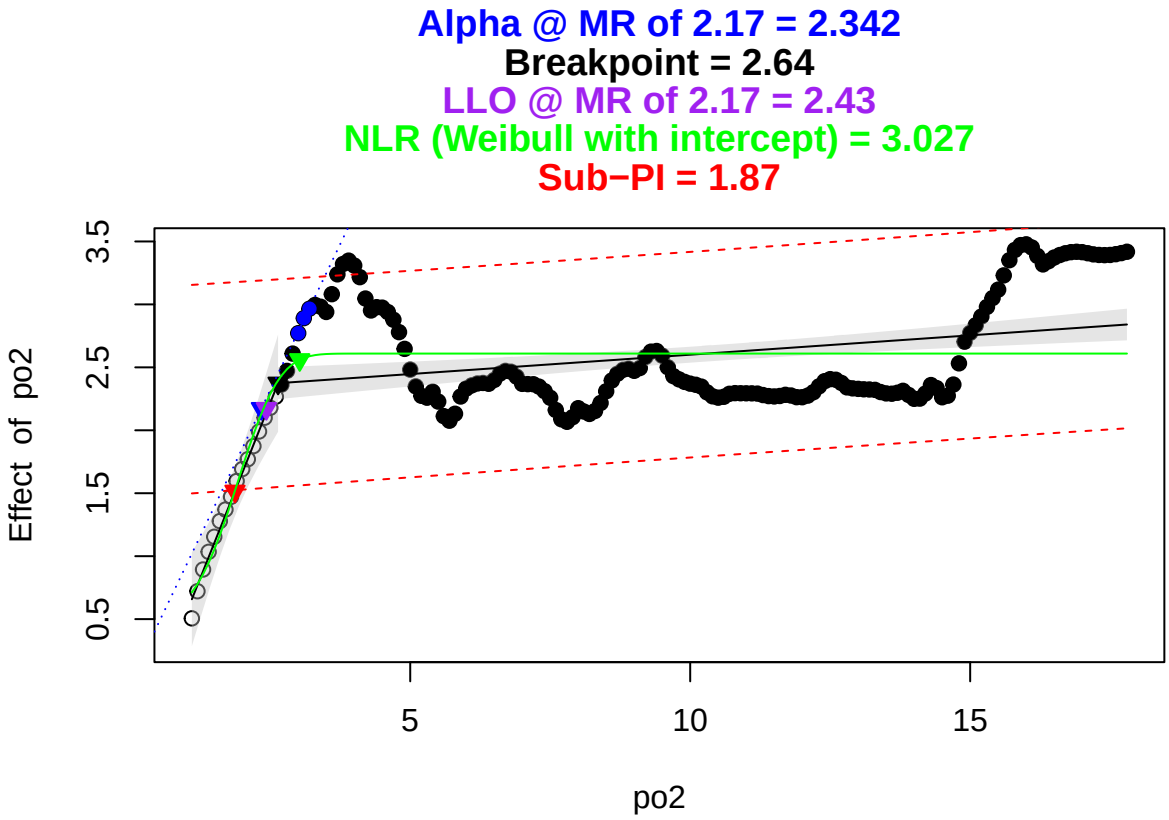

Gr3 Muus 1800−2 pcrit 07−28−21.txt
mass= 16.9
resp vol=1
drop time=2.8 hr
O2 drop=192.1
rough mean MO2=4.1
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
10000
pcrit.raw$times

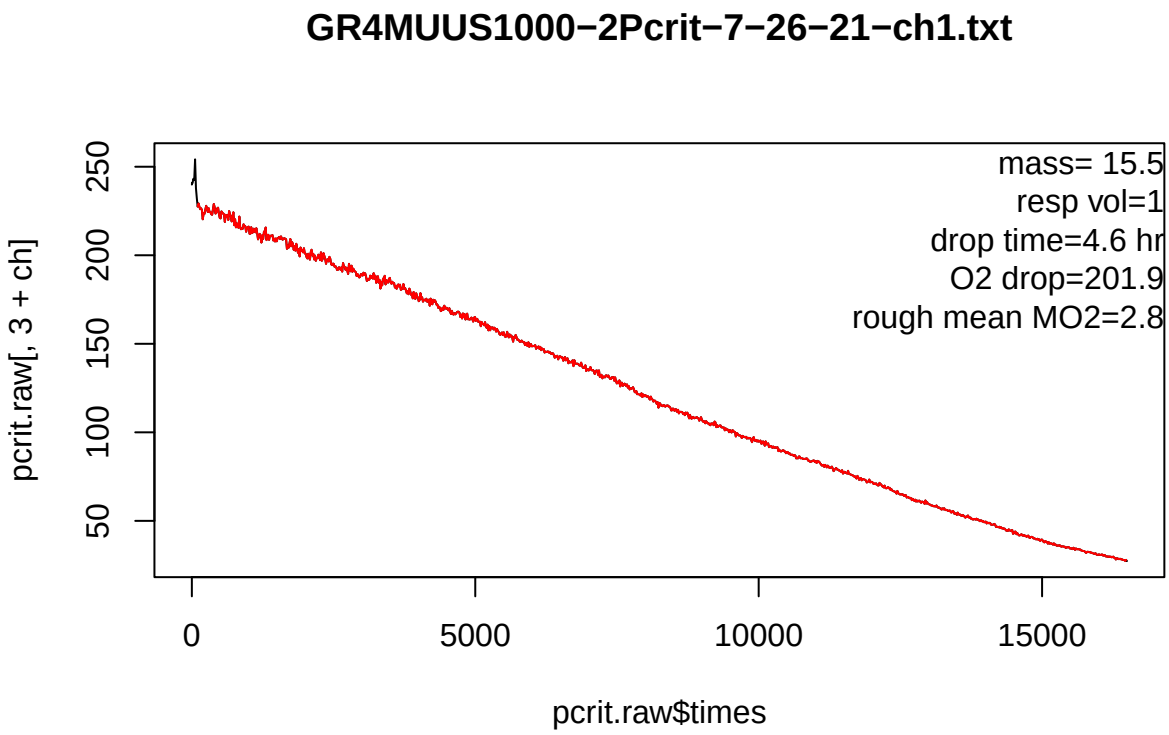

Alpha @ MR of 2.16 = 1.542
Breakpoint = 2.959
LLO @ MR of 2.16 = 1.983
NLR (Pareto) = 3.857
Sub−PI = 2.78
Effect of po2
po2
1
2
3
4
5
5
10
15

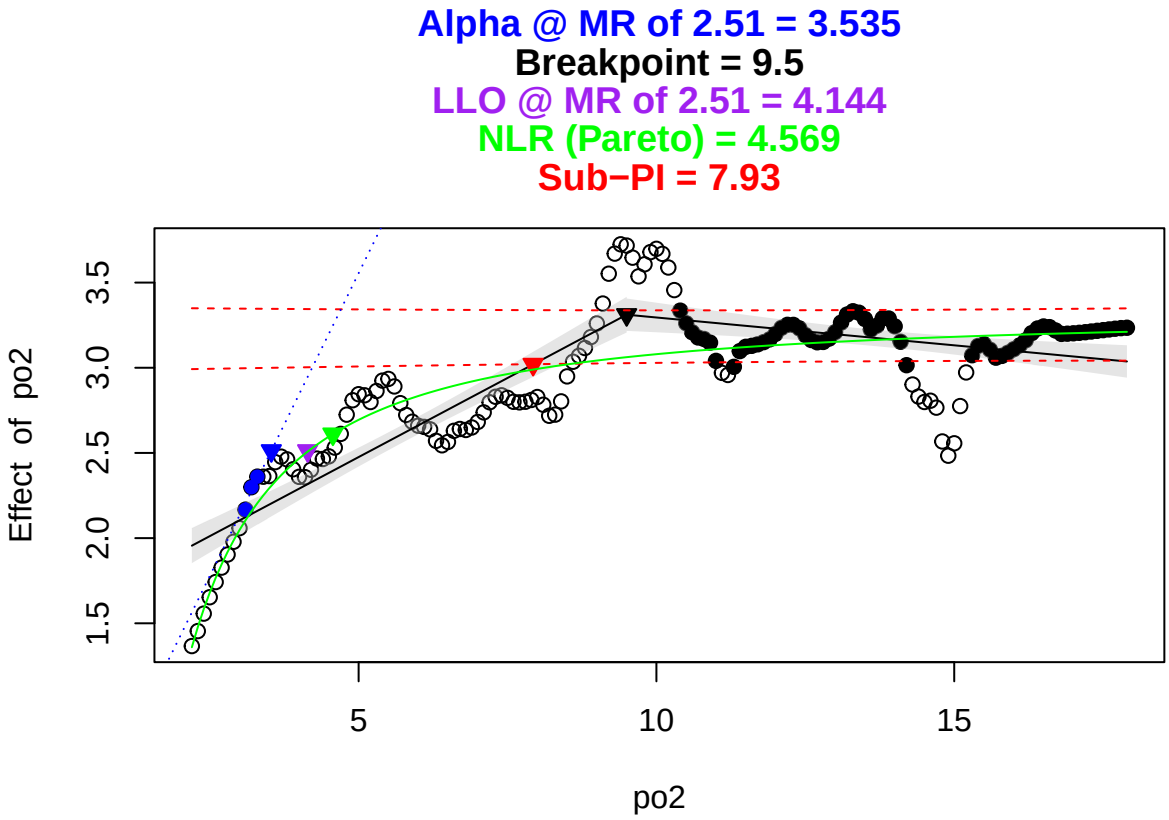

Gr3 Muus 1800−2 pcrit 08−03−21.txt
mass= 16.9
resp vol=1
drop time=5.6 hr
O2 drop=236.9
rough mean MO2=2.5
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
20000
pcrit.raw$times

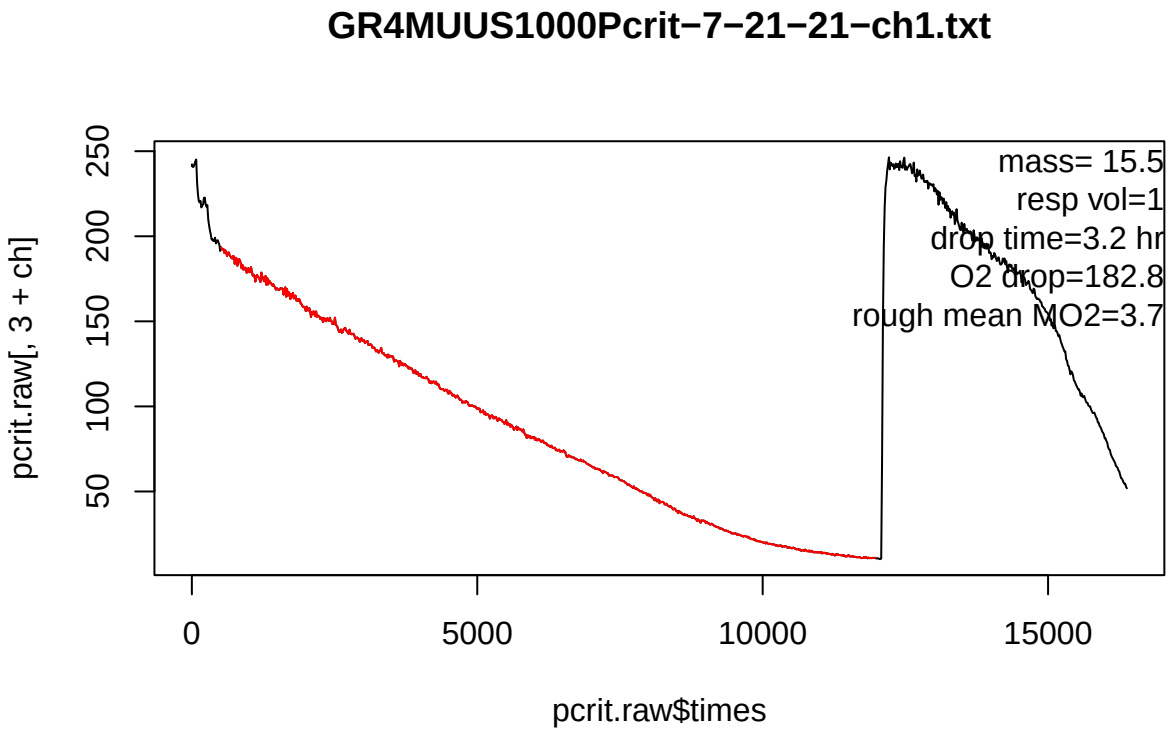

Alpha @ MR of 2.16 = 3.363
Breakpoint = 14.254
LLO @ MR of 2.16 = 8.734
NLR (Power) = 16.472
Sub−PI = 18.1
Effect of po2
1
2
3
4
5
6
5
10
15
po2

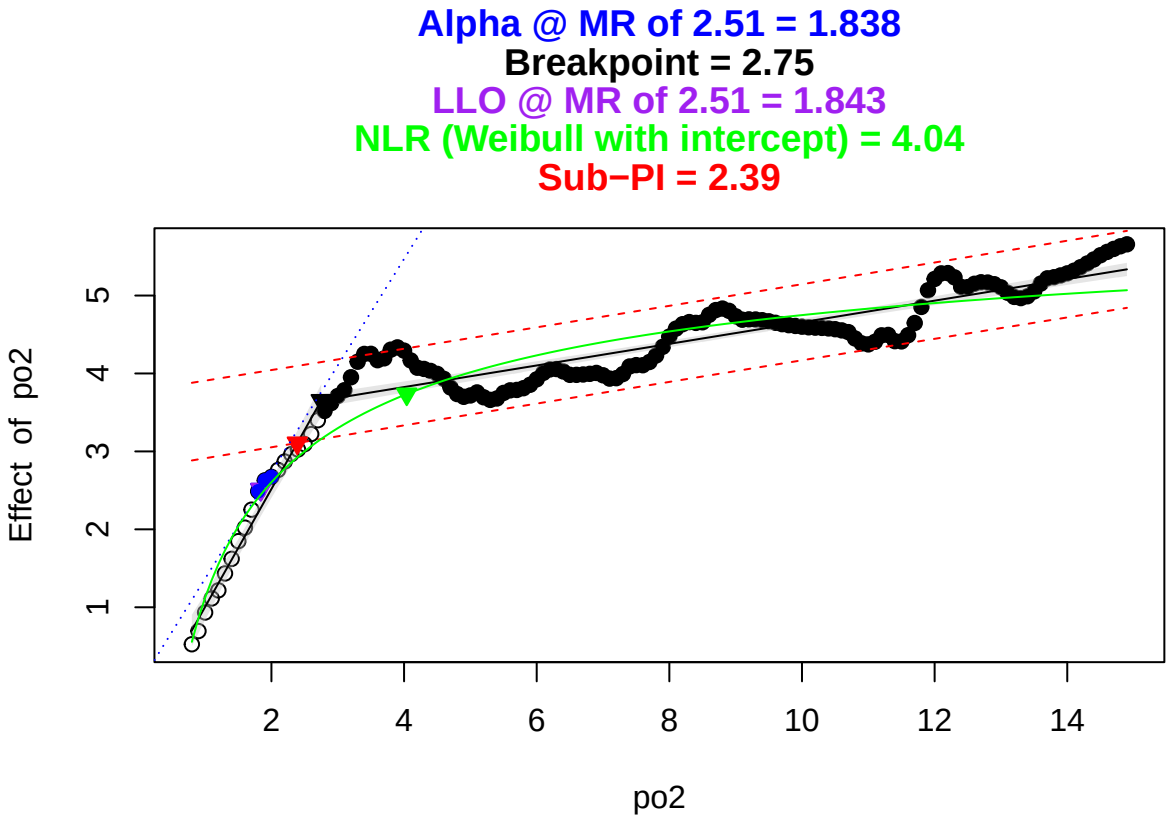

Gr3 Muus1000−2 7 day pcrit 7−27−21.txt
mass= 21.6
resp vol=1
drop time=4.3 hr
O2 drop=213.6
rough mean MO2=2.3
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
pcrit.raw$times

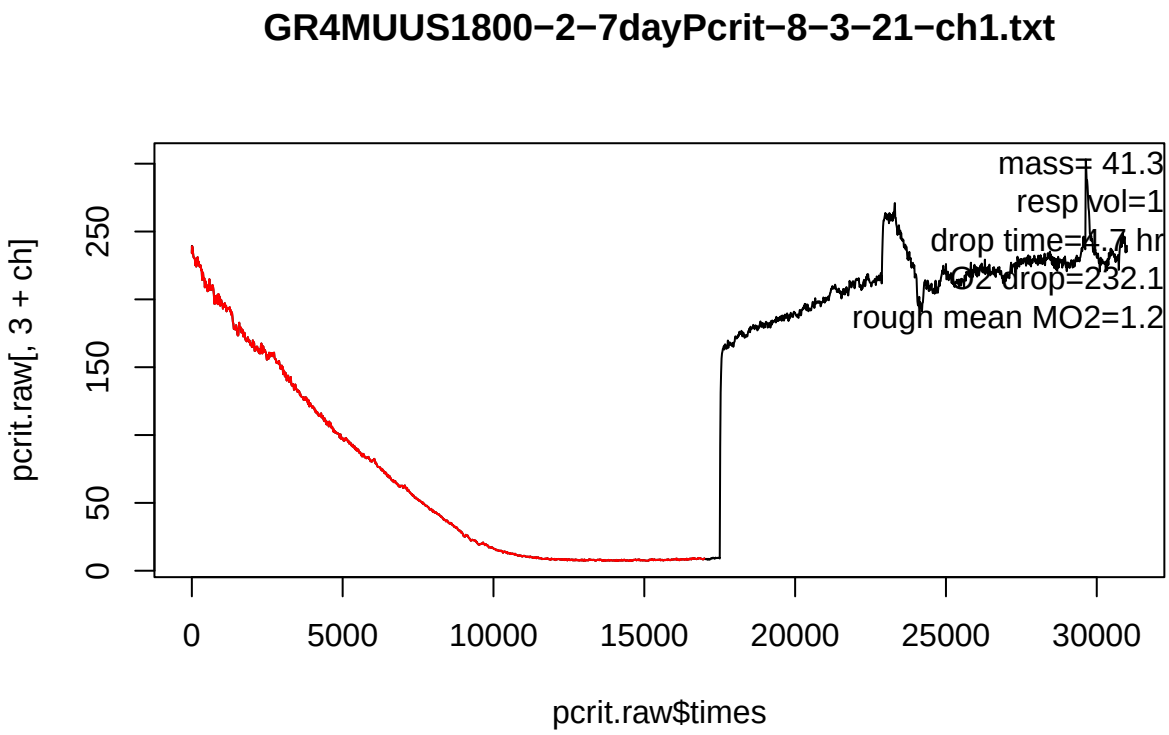

Alpha @ MR of 2.17 = 2.342
Breakpoint = 2.64
LLO @ MR of 2.17 = 2.43
NLR (Weibull with intercept) = 3.027
Sub−PI = 1.87
Effect of po2
0.5
1.5
2.5
3.5
5
10
15
po2

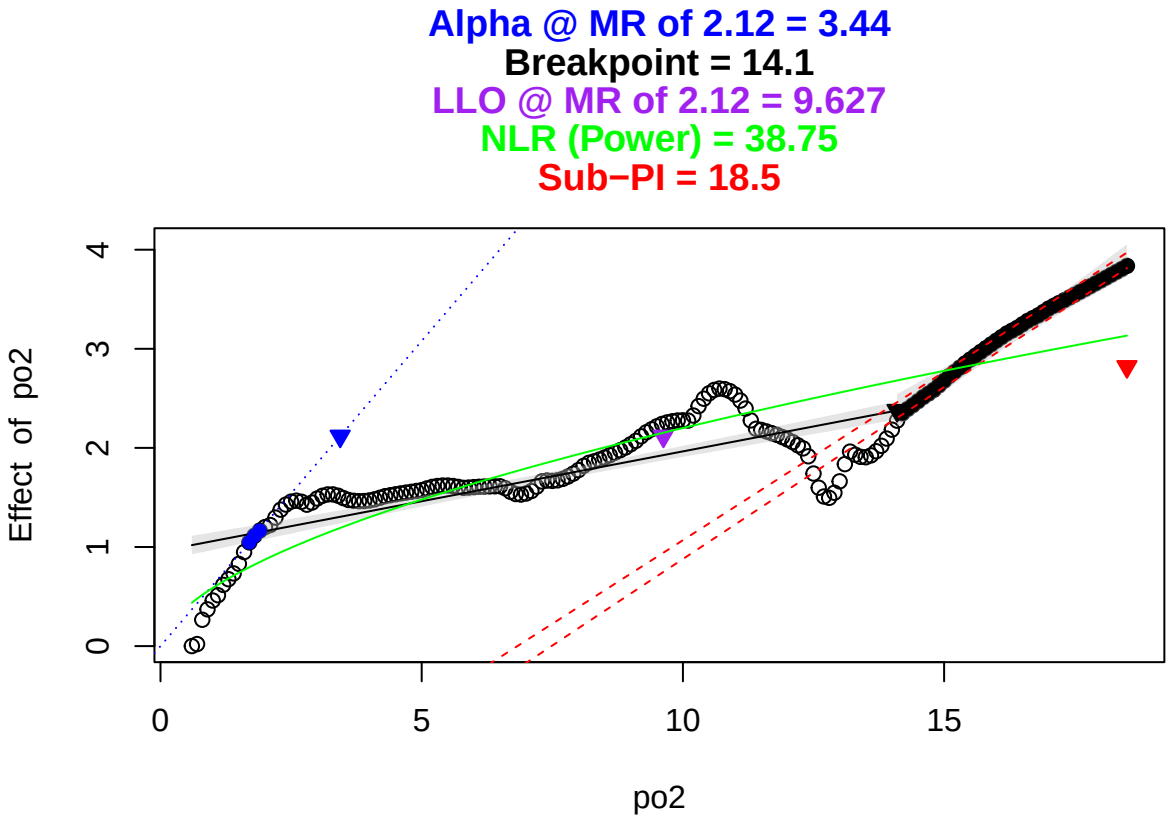

GR4MUUS1000−2Pcrit−7−26−21−ch1.txt
mass= 15.5
resp vol=1
drop time=4.6 hr
O2 drop=201.9
rough mean MO2=2.8
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
pcrit.raw$times

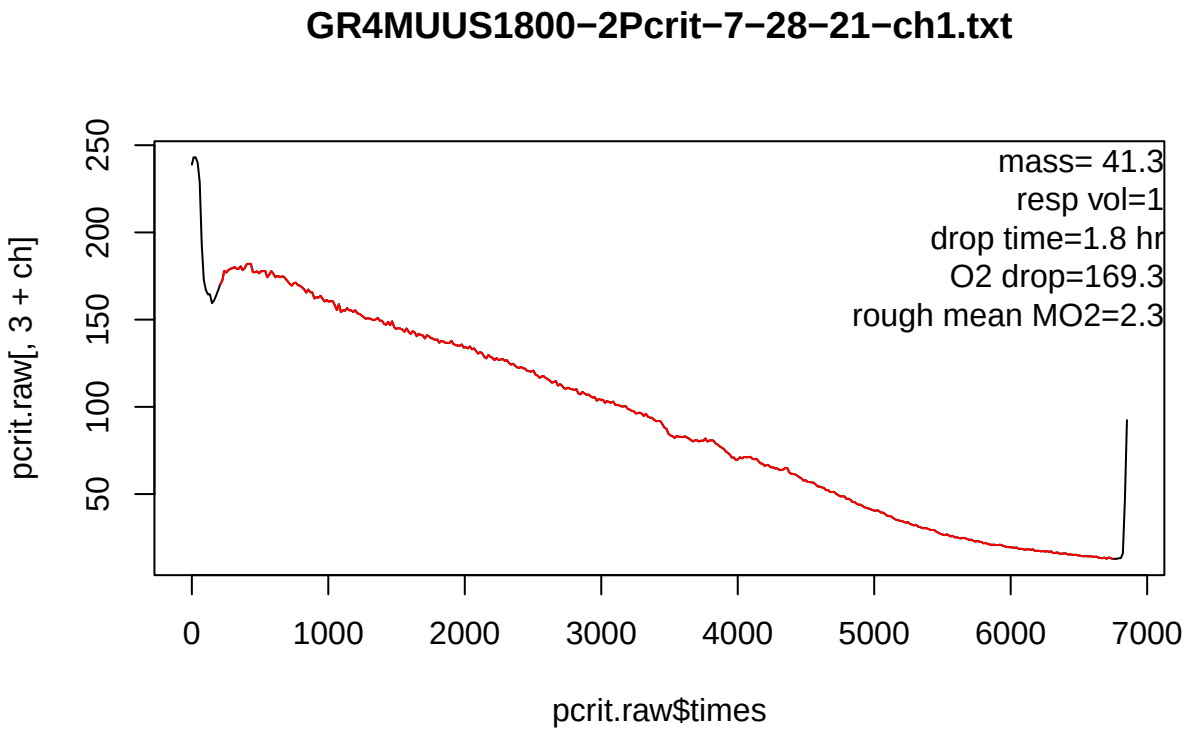

Alpha @ MR of 2.51 = 3.535
Breakpoint = 9.5
LLO @ MR of 2.51 = 4.144
NLR (Pareto) = 4.569
Sub−PI = 7.93
Effect of po2
1.5
2.0
2.5
3.0
3.5
5
10
15
po2

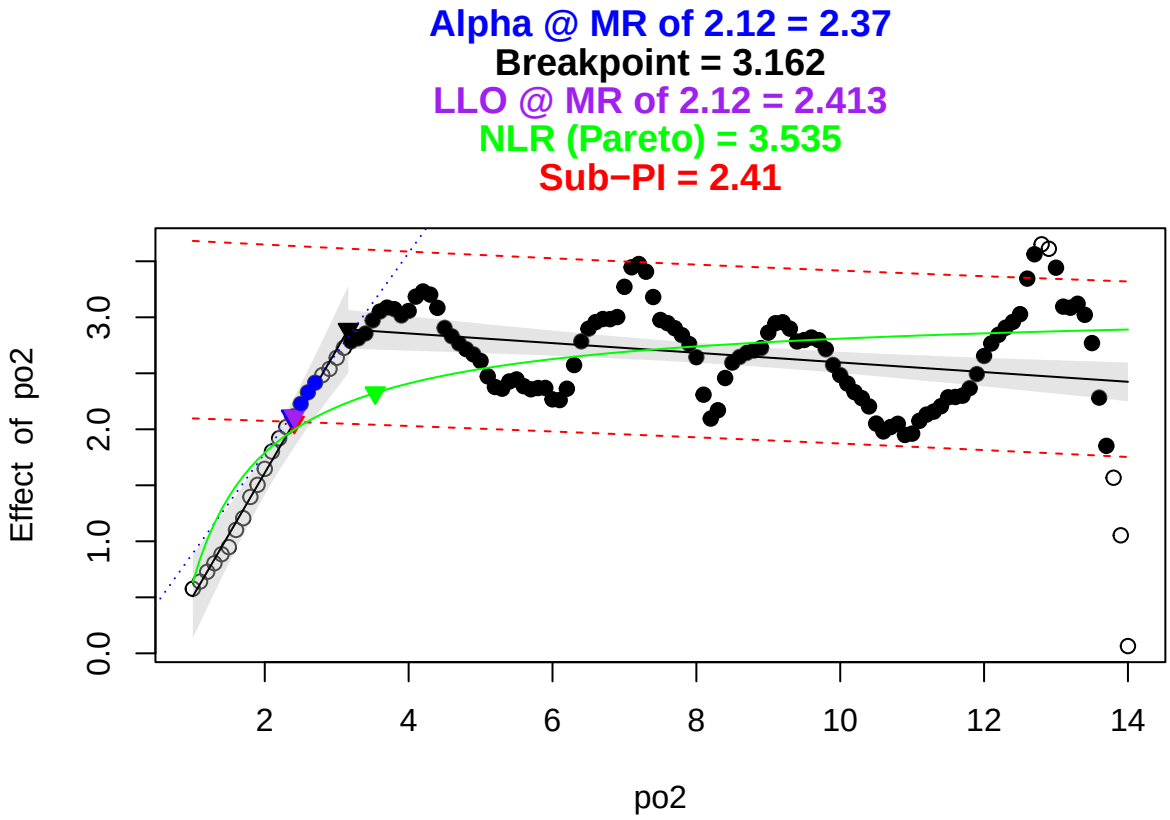

GR4MUUS1000Pcrit−7−21−21−ch1.txt
mass= 15.5
resp vol=1
drop time=3.2 hr
O2 drop=182.8
rough mean MO2=3.7
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
pcrit.raw$times

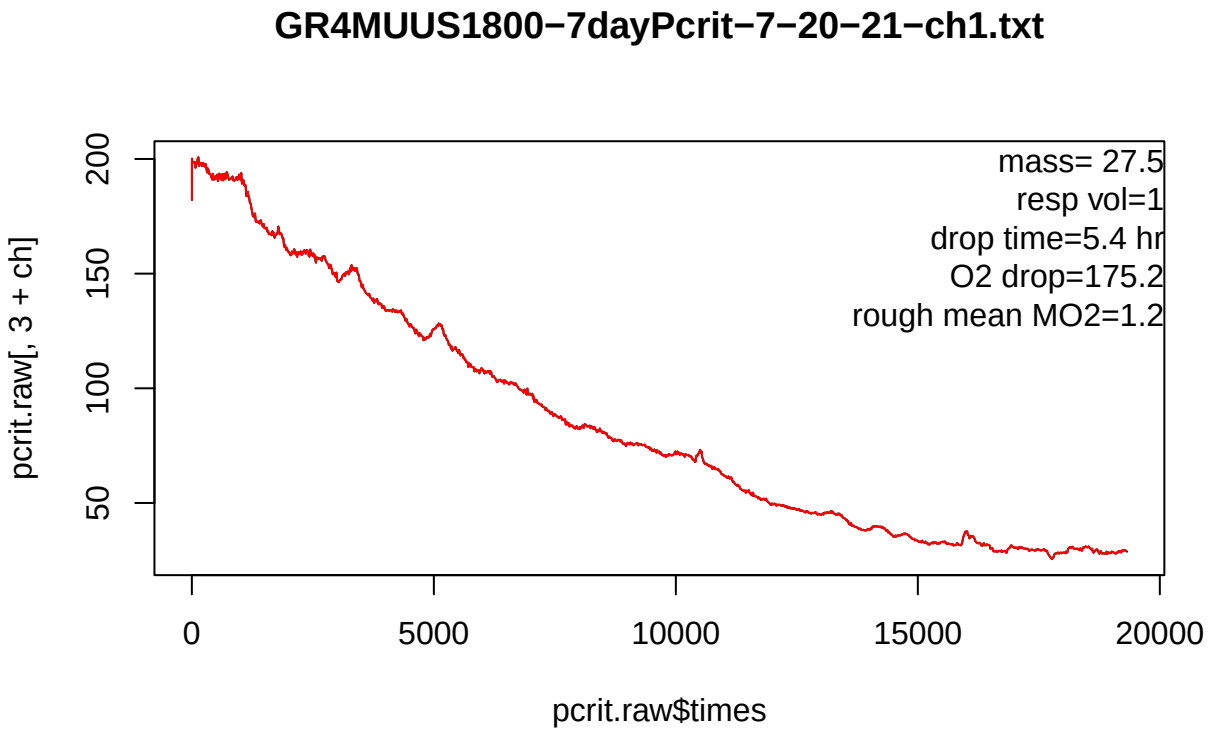

Alpha @ MR of 2.51 = 1.838
Breakpoint = 2.75
LLO @ MR of 2.51 = 1.843
NLR (Weibull with intercept) = 4.04
Sub−PI = 2.39
Effect of po2
1
2
3
4
5
2
4
6
8
10
12
14
po2

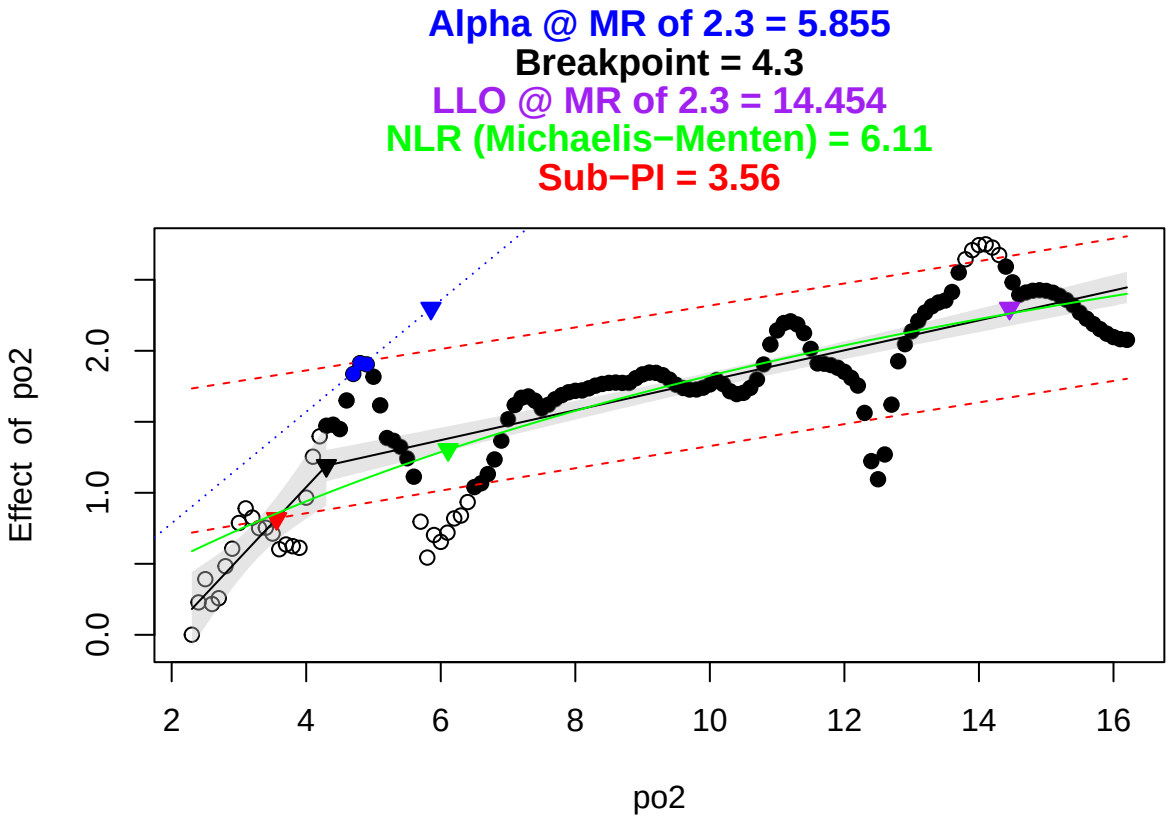

GR4MUUS1800−2−7dayPcrit−8−3−21−ch1.txt
mass= 41.3
resp vol=1
drop time=4.7 hr
O2 drop=232.1
rough mean MO2=1.2
pcrit.raw[, 3 + ch]
0
50
150
250
0
5000
10000
15000
20000
25000
30000
pcrit.raw$times

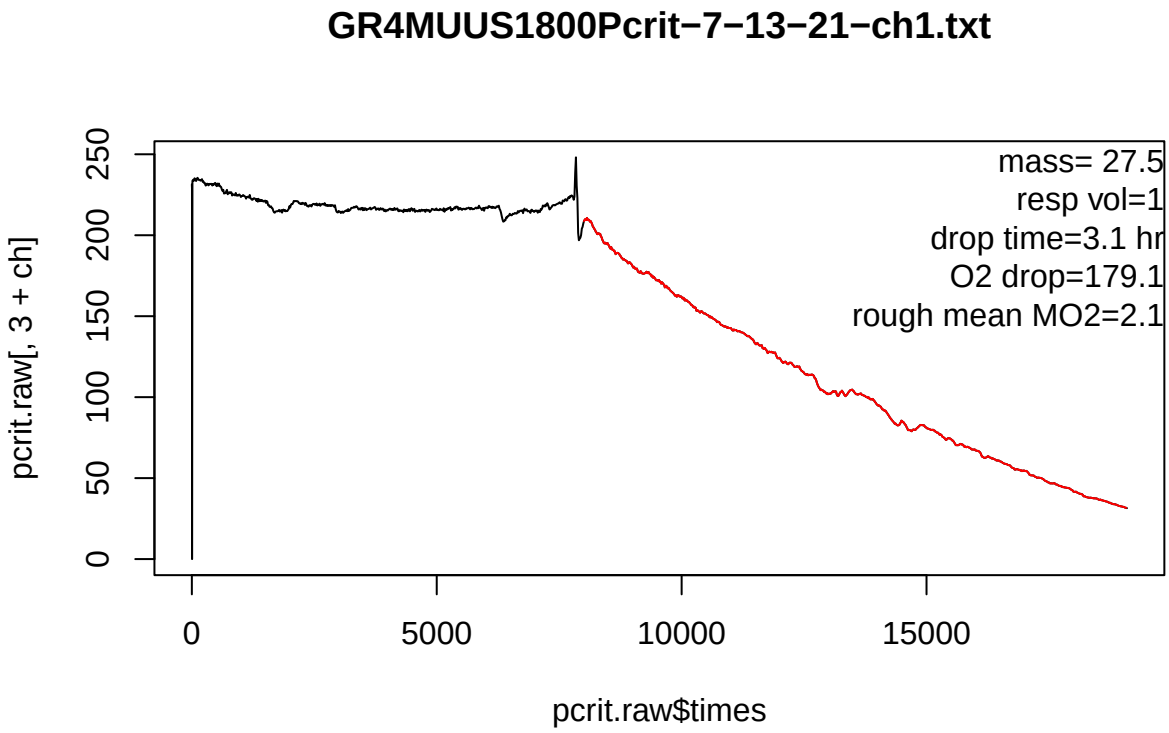

Alpha @ MR of 2.12 = 3.44
Breakpoint = 14.1
LLO @ MR of 2.12 = 9.627
NLR (Power) = 38.75
Sub−PI = 18.5
Effect of po2
0
1
2
3
4
0
5
10
15
po2

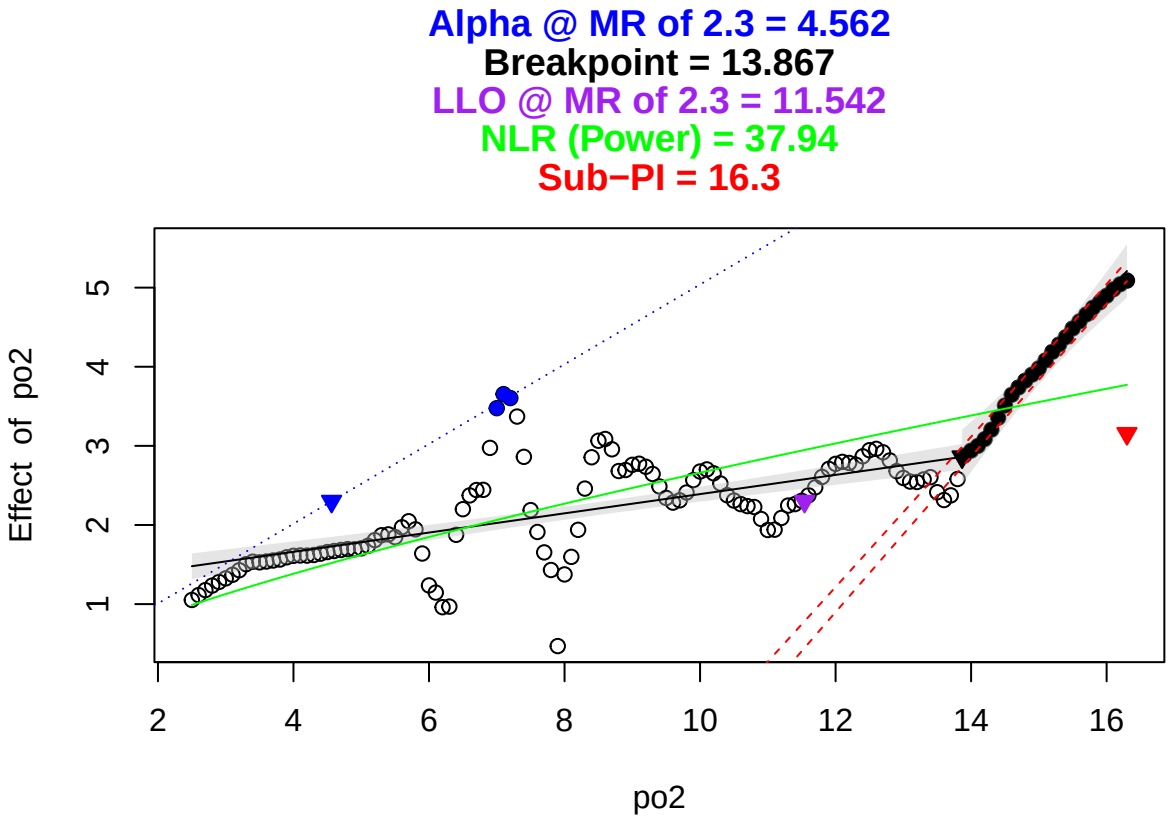

GR4MUUS1800−2Pcrit−7−28−21−ch1.txt
mass= 41.3
resp vol=1
drop time=1.8 hr
O2 drop=169.3
rough mean MO2=2.3
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
1000
2000
3000
4000
5000
6000
7000
pcrit.raw$times

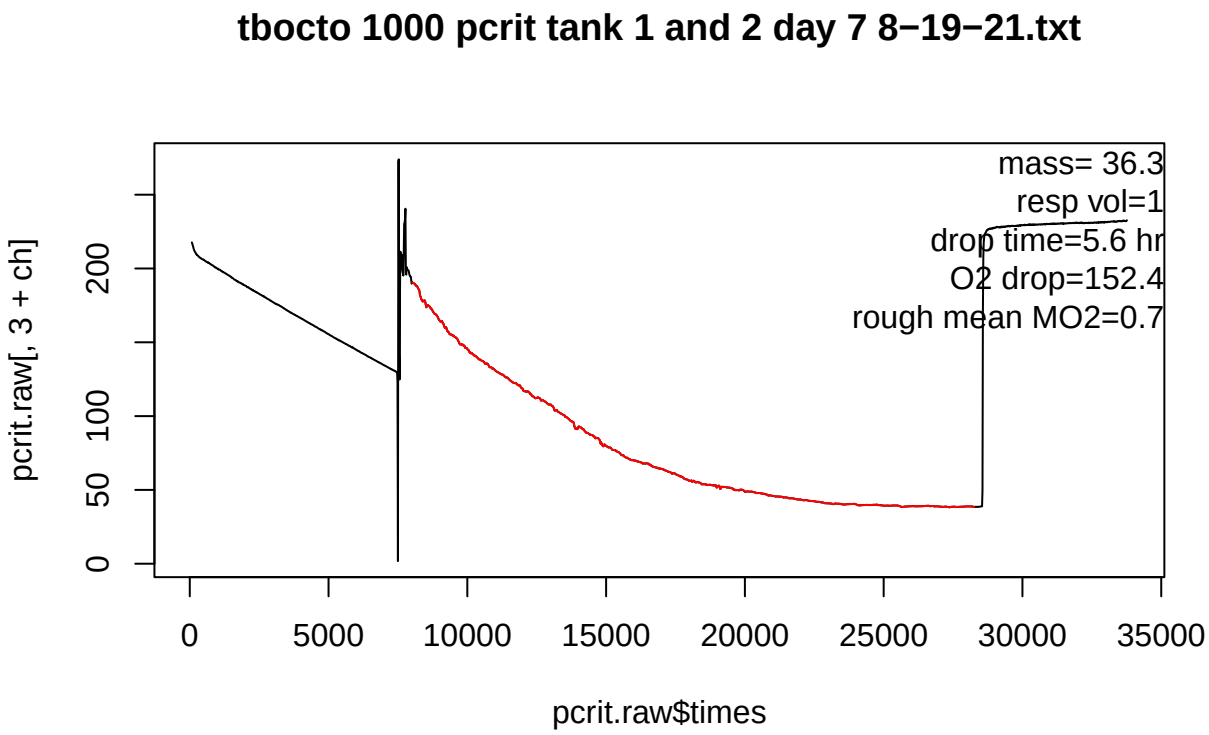

Alpha @ MR of 2.12 = 2.37
Breakpoint = 3.162
LLO @ MR of 2.12 = 2.413
NLR (Pareto) = 3.535
Sub−PI = 2.41
Effect of po2
0.0
1.0
2.0
3.0
2
4
6
8
10
12
14
po2

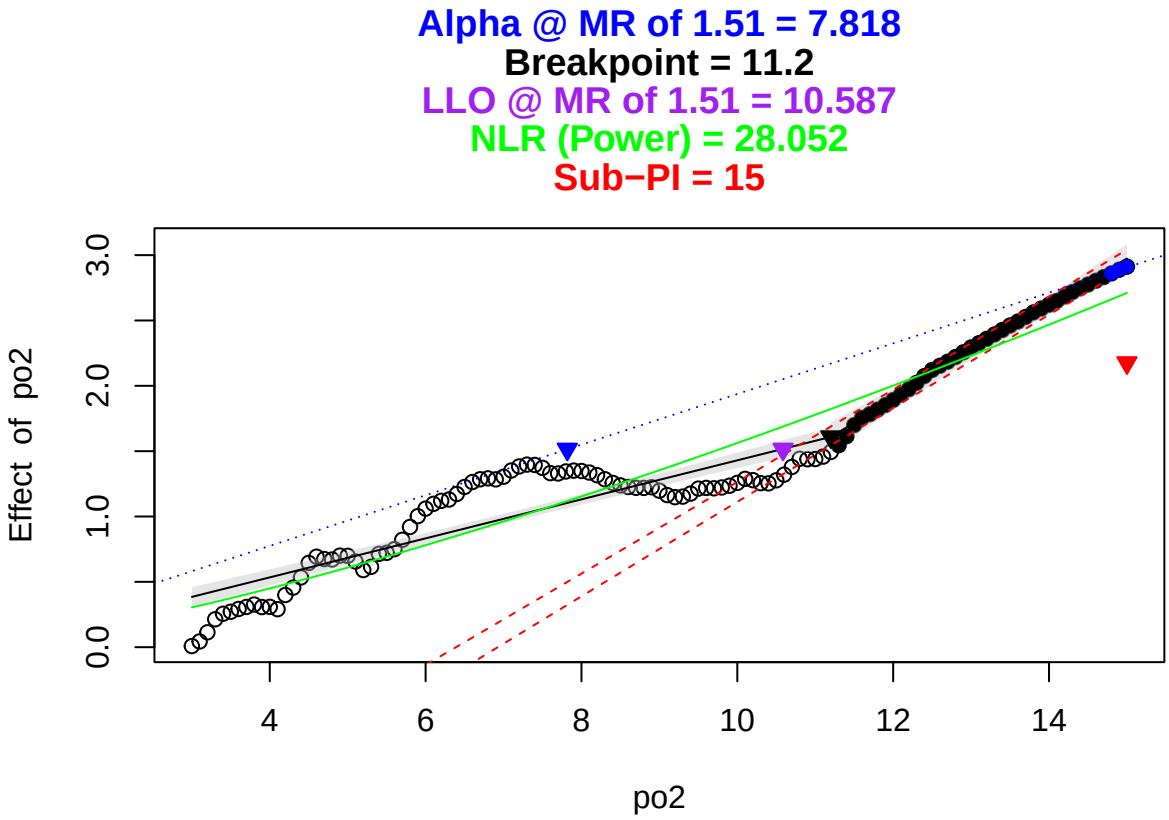

GR4MUUS1800−7dayPcrit−7−20−21−ch1.txt
mass= 27.5
resp vol=1
drop time=5.4 hr
O2 drop=175.2
rough mean MO2=1.2
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
pcrit.raw$times

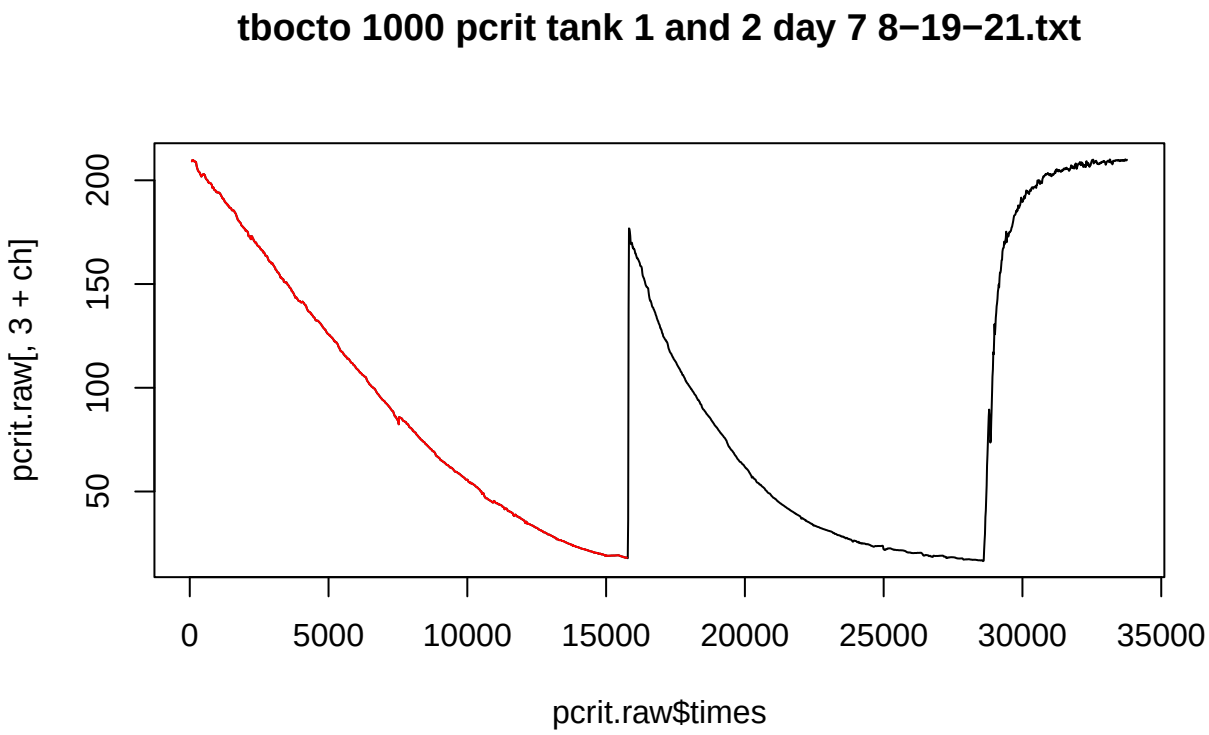

Alpha @ MR of 2.3 = 5.855
Breakpoint = 4.3
LLO @ MR of 2.3 = 14.454
NLR (Michaelis−Menten) = 6.11
Sub−PI = 3.56
Effect of po2
0.0
1.0
2.0
2
4
6
8
10
12
14
16
po2

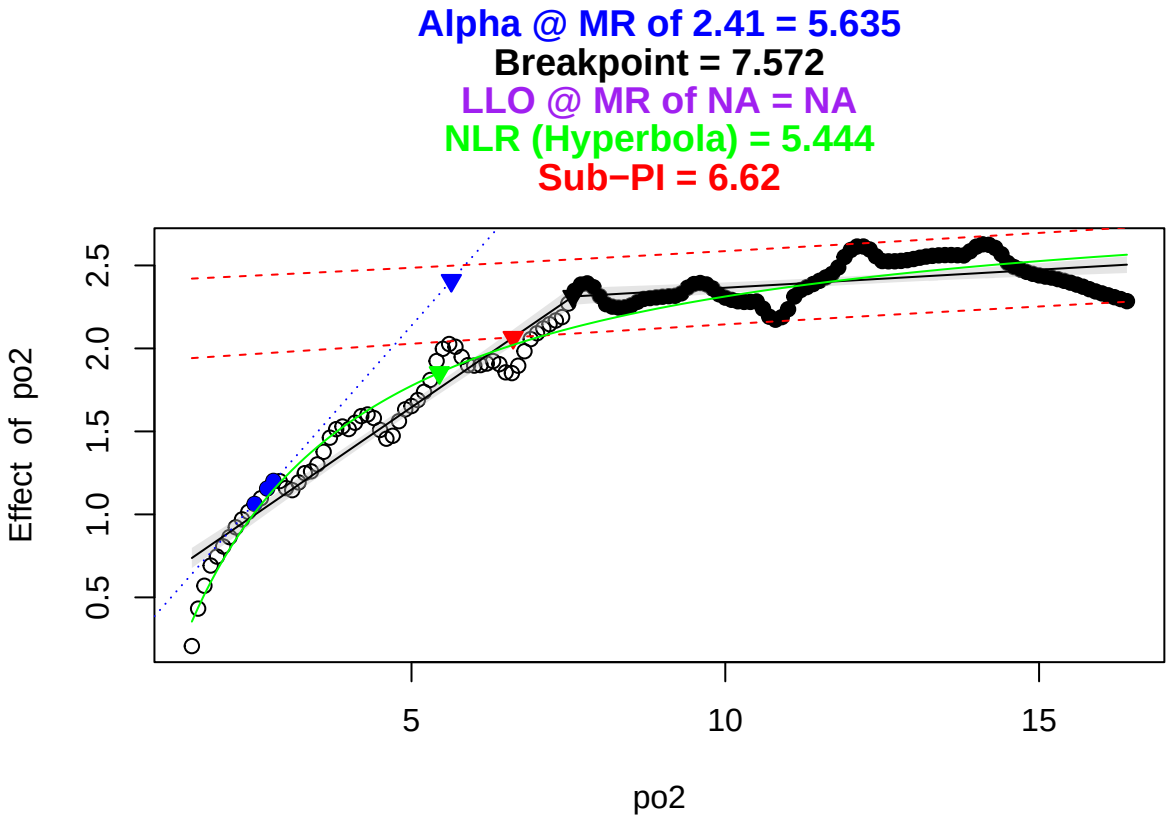

GR4MUUS1800Pcrit−7−13−21−ch1.txt
mass= 27.5
resp vol=1
drop time=3.1 hr
O2 drop=179.1
rough mean MO2=2.1
pcrit.raw[, 3 + ch]
0
50
100
150
200
250
0
5000
10000
15000
pcrit.raw$times

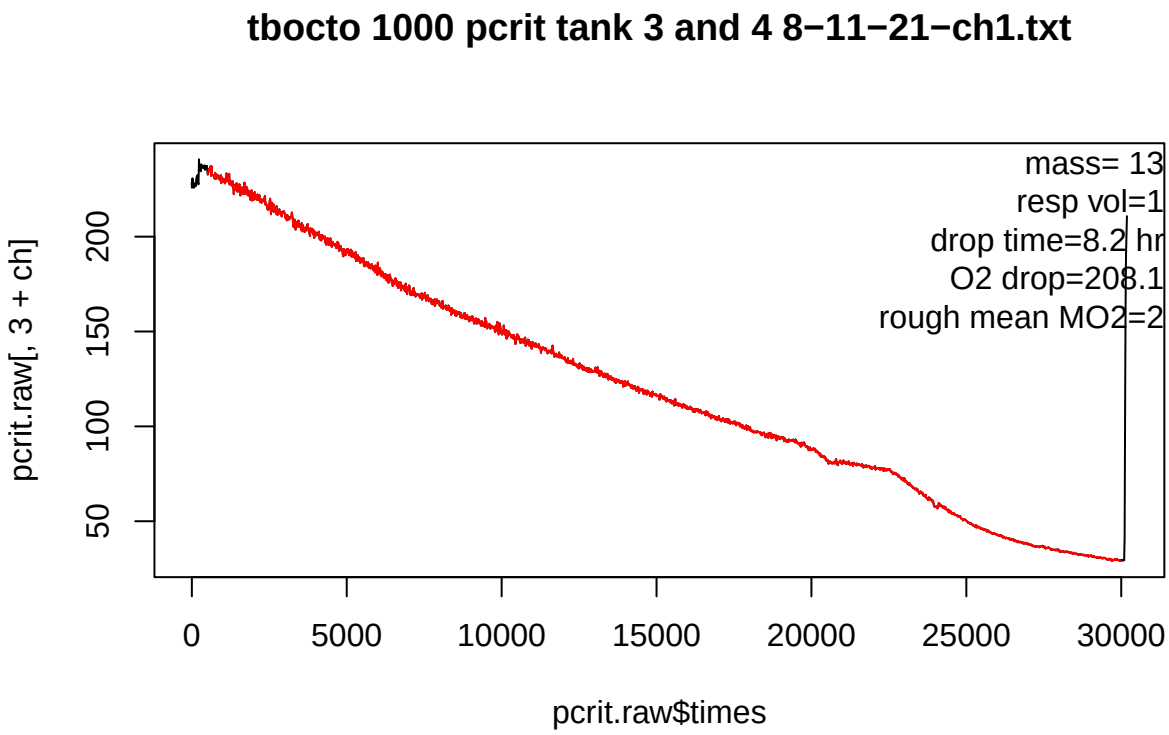

Alpha @ MR of 2.3 = 4.562
Breakpoint = 13.867
LLO @ MR of 2.3 = 11.542
NLR (Power) = 37.94
Sub−PI = 16.3
Effect of po2
po2
1
2
3
4
5
2
4
6
8
10
12
14
16

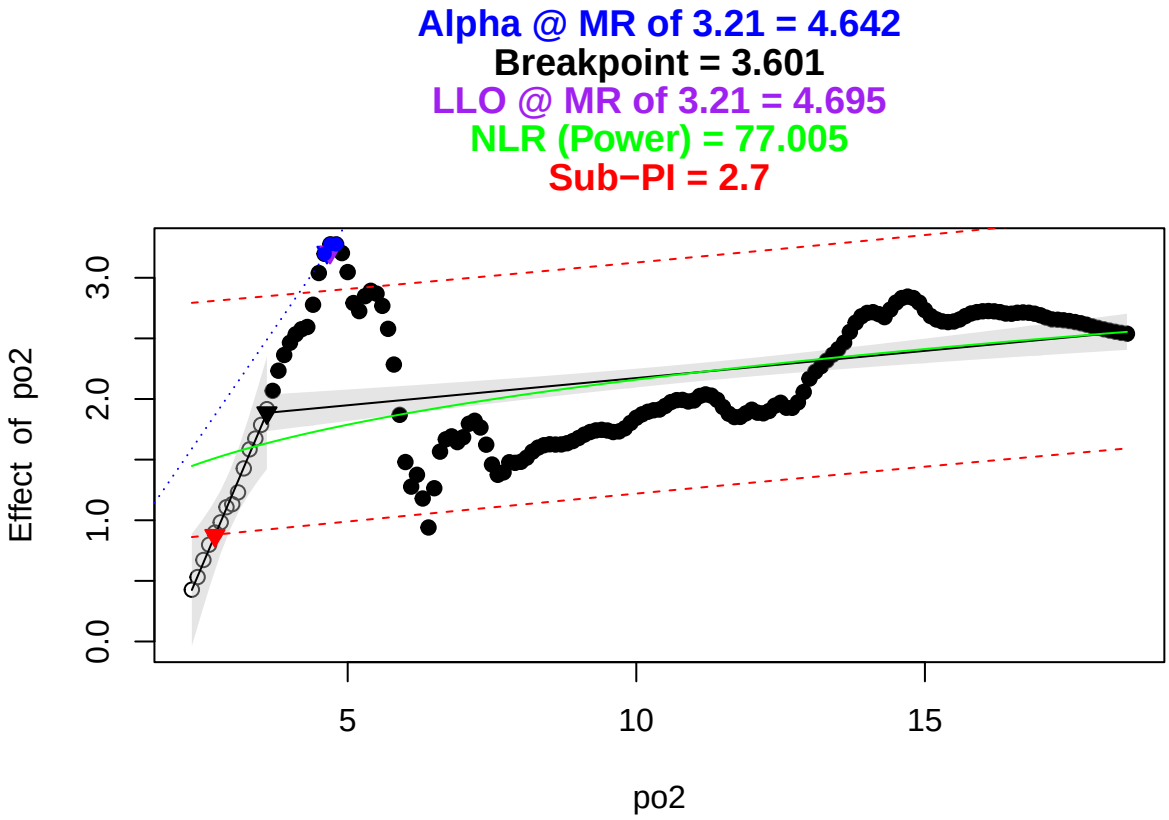

tbocto 1000 pcrit tank 1 and 2 day 7 8−19−21.txt
mass= 36.3
resp vol=1
drop time=5.6 hr
O2 drop=152.4
rough mean MO2=0.7
pcrit.raw[, 3 + ch]
0
50
100
200
0
5000
10000
15000
20000
25000
30000
35000
pcrit.raw$times

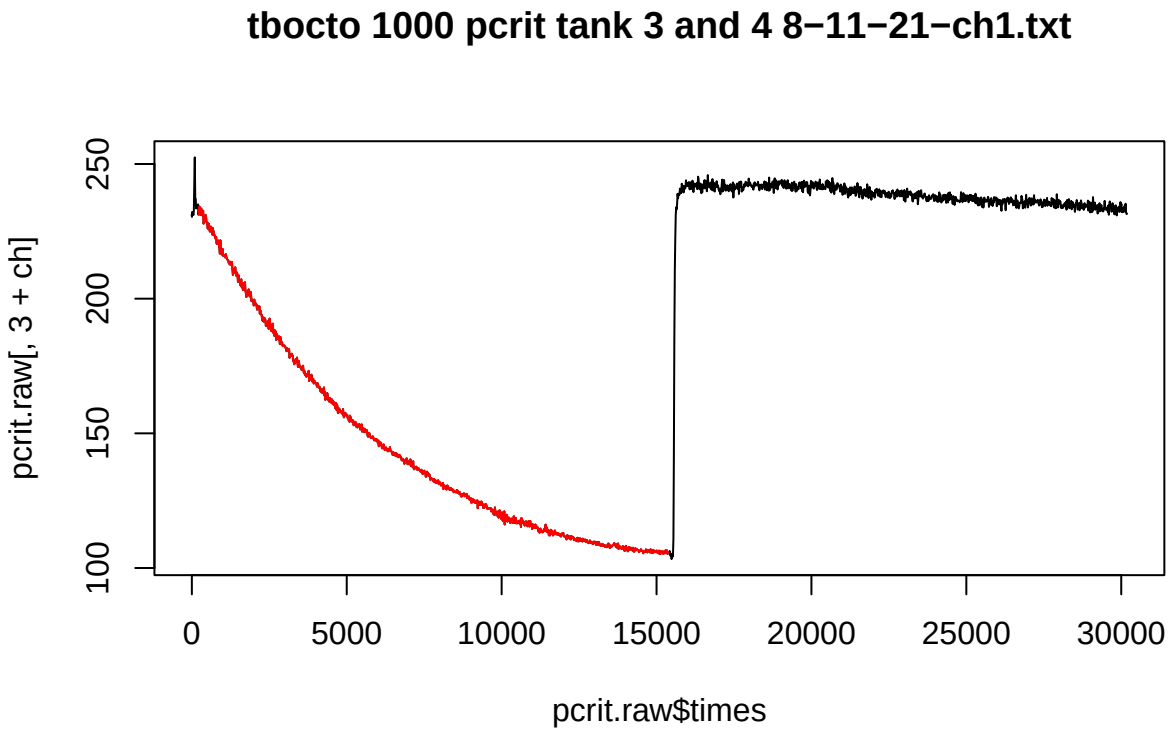

Alpha @ MR of 1.51 = 7.818
Breakpoint = 11.2
LLO @ MR of 1.51 = 10.587
NLR (Power) = 28.052
Sub−PI = 15
Effect of po2
0.0
1.0
2.0
3.0
4
6
8
10
12
14
po2

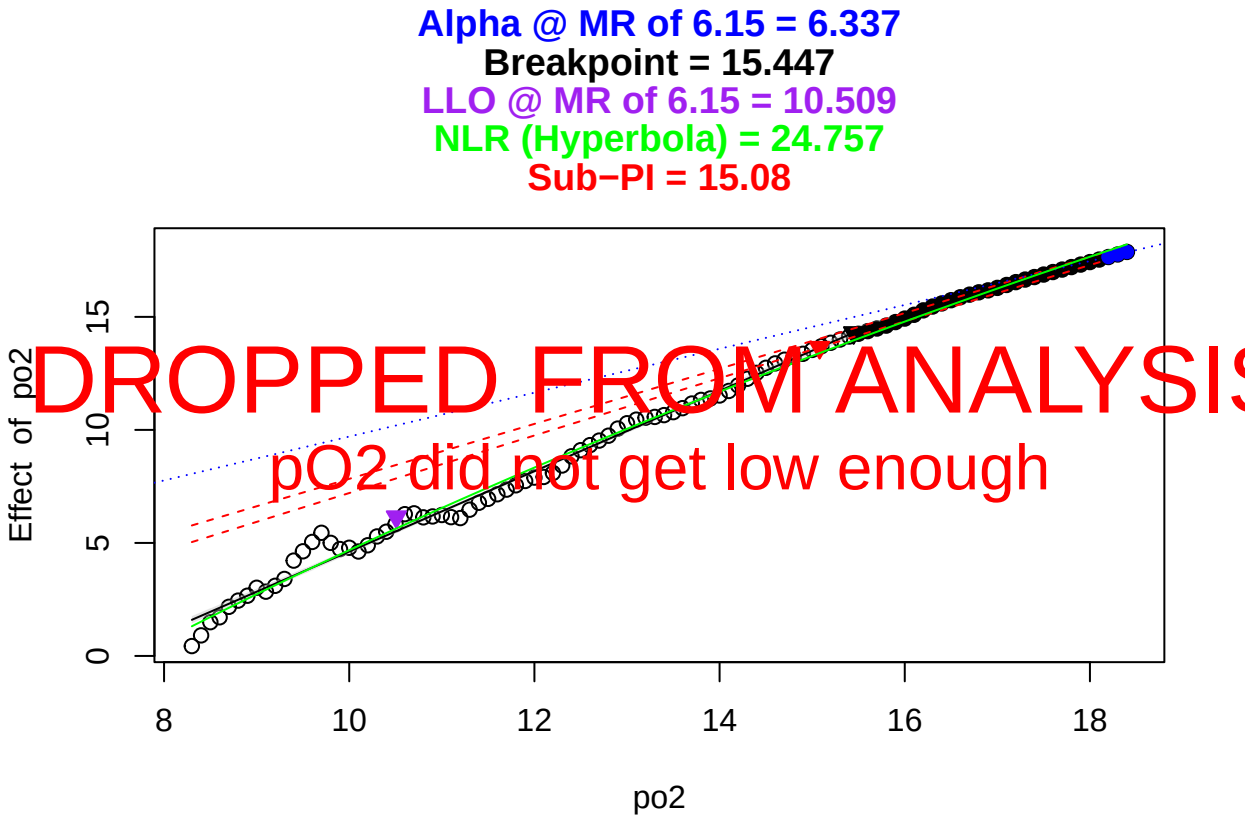

tbocto 1000 pcrit tank 1 and 2 day 7 8−19−21.txt
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
25000
30000
35000
pcrit.raw$times

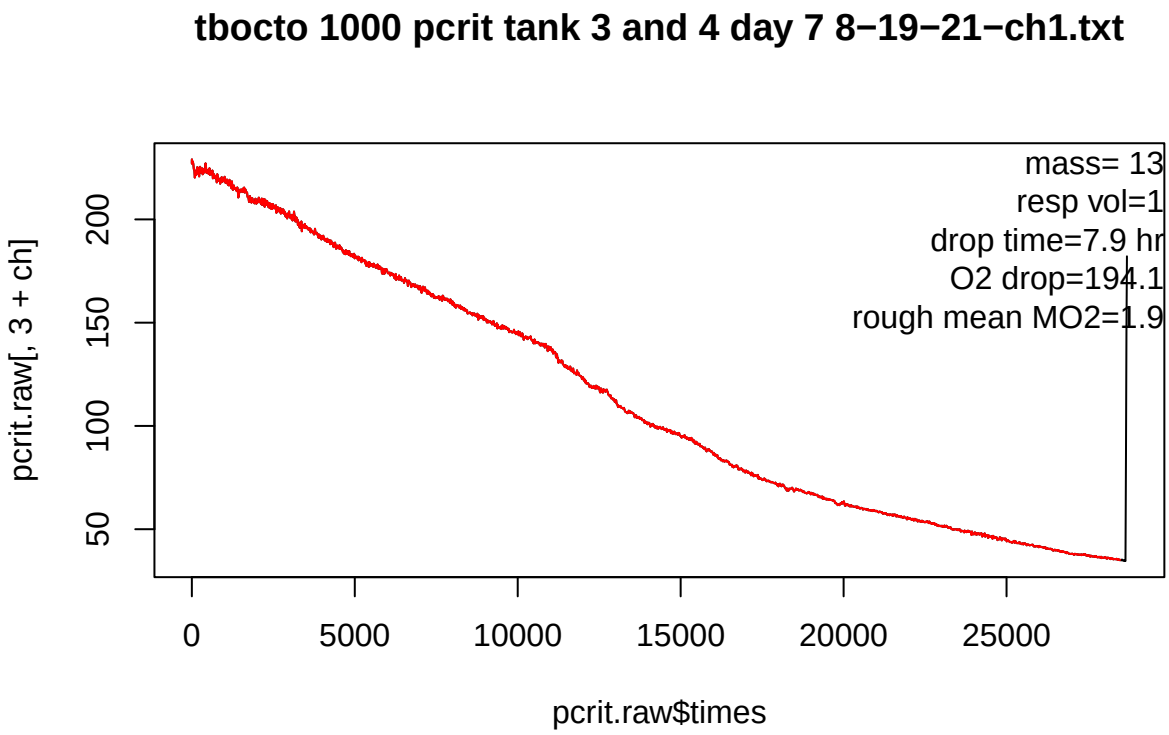

Alpha @ MR of 2.41 = 5.635
Breakpoint = 7.572
LLO @ MR of NA = NA
NLR (Hyperbola) = 5.444
Sub−PI = 6.62
Effect of po2
0.5
1.0
1.5
2.0
2.5
5
10
15
po2

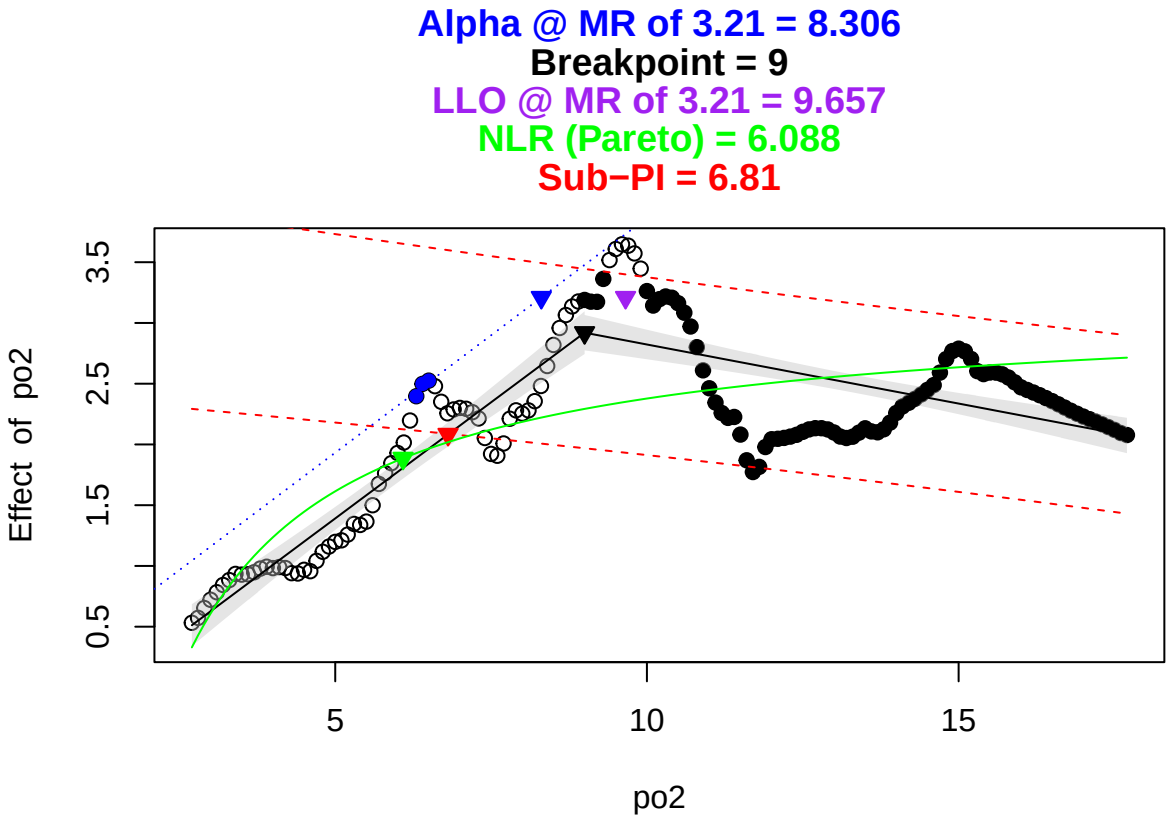

tbocto 1000 pcrit tank 3 and 4 8−11−21−ch1.txt
mass= 13
resp vol=1
drop time=8.2 hr
O2 drop=208.1
rough mean MO2=2
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
25000
30000
pcrit.raw$times

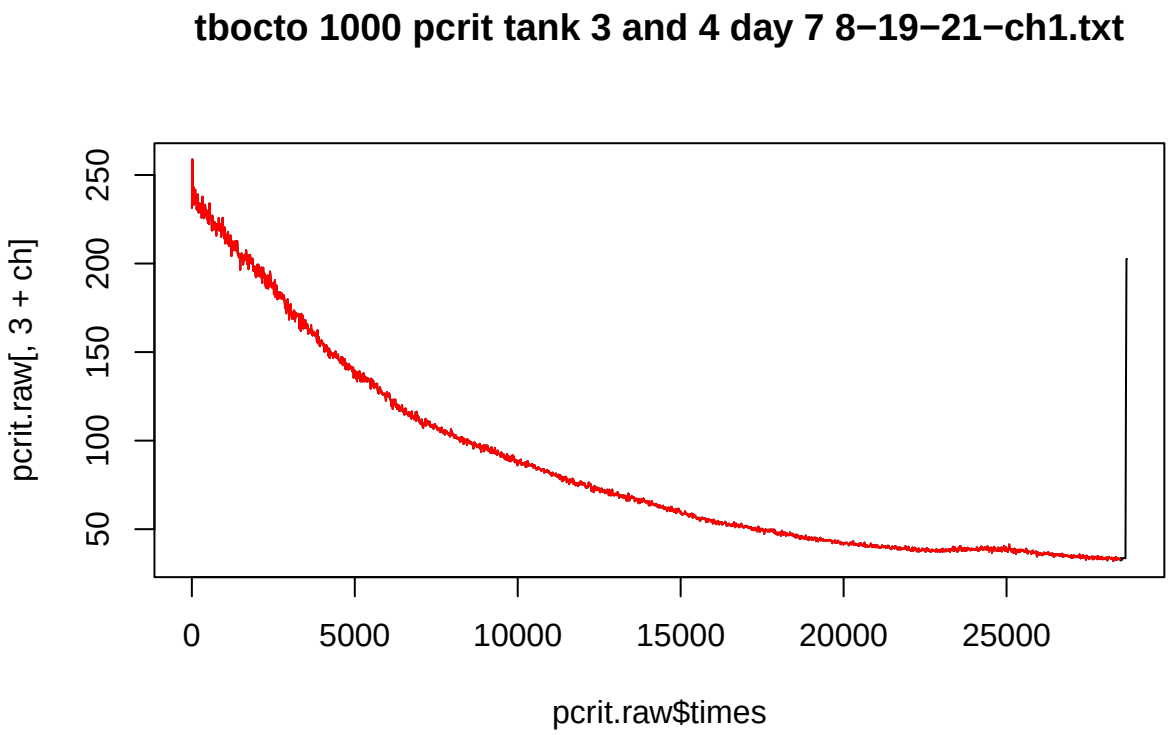

Alpha @ MR of 3.21 = 4.642
Breakpoint = 3.601
LLO @ MR of 3.21 = 4.695
NLR (Power) = 77.005
Sub−PI = 2.7
Effect of po2
0.0
1.0
2.0
3.0
5
10
15
po2

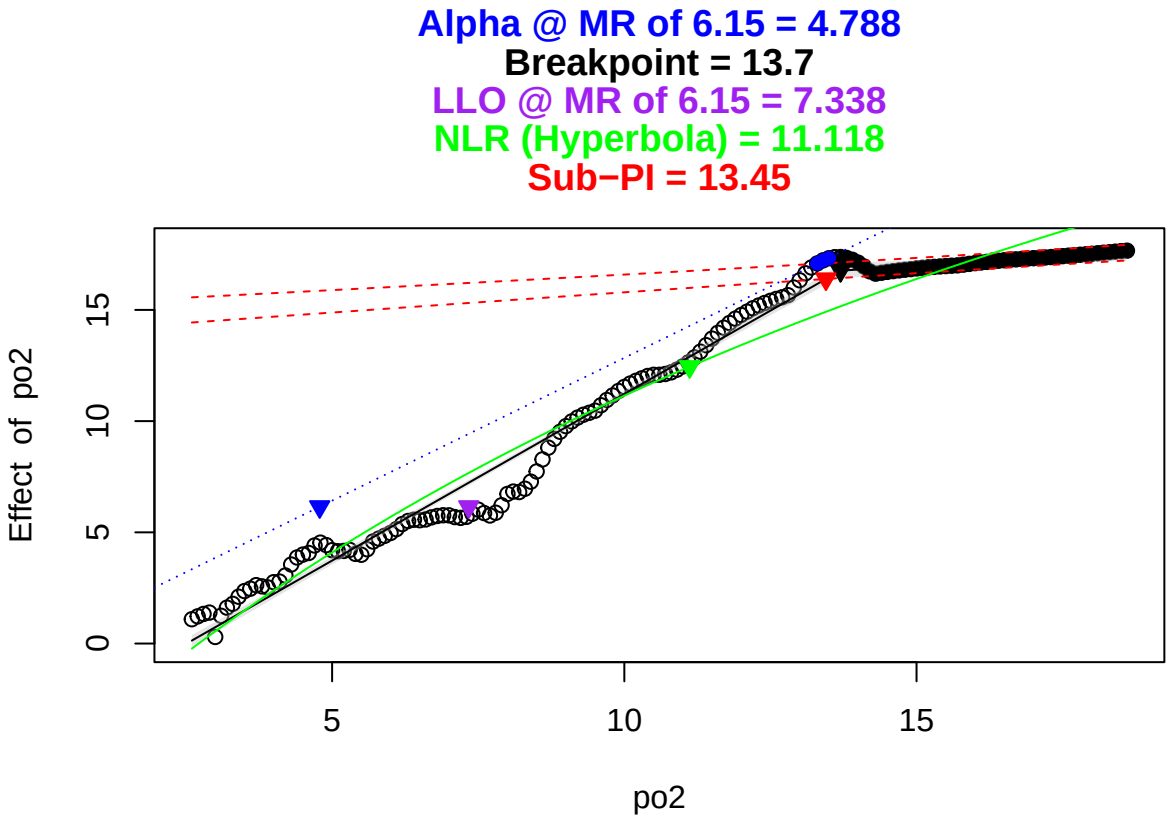

tbocto 1000 pcrit tank 3 and 4 8−11−21−ch1.txt
pcrit.raw[, 3 + ch]
100
150
200
250
0
5000
10000
15000
20000
25000
30000
pcrit.raw$times

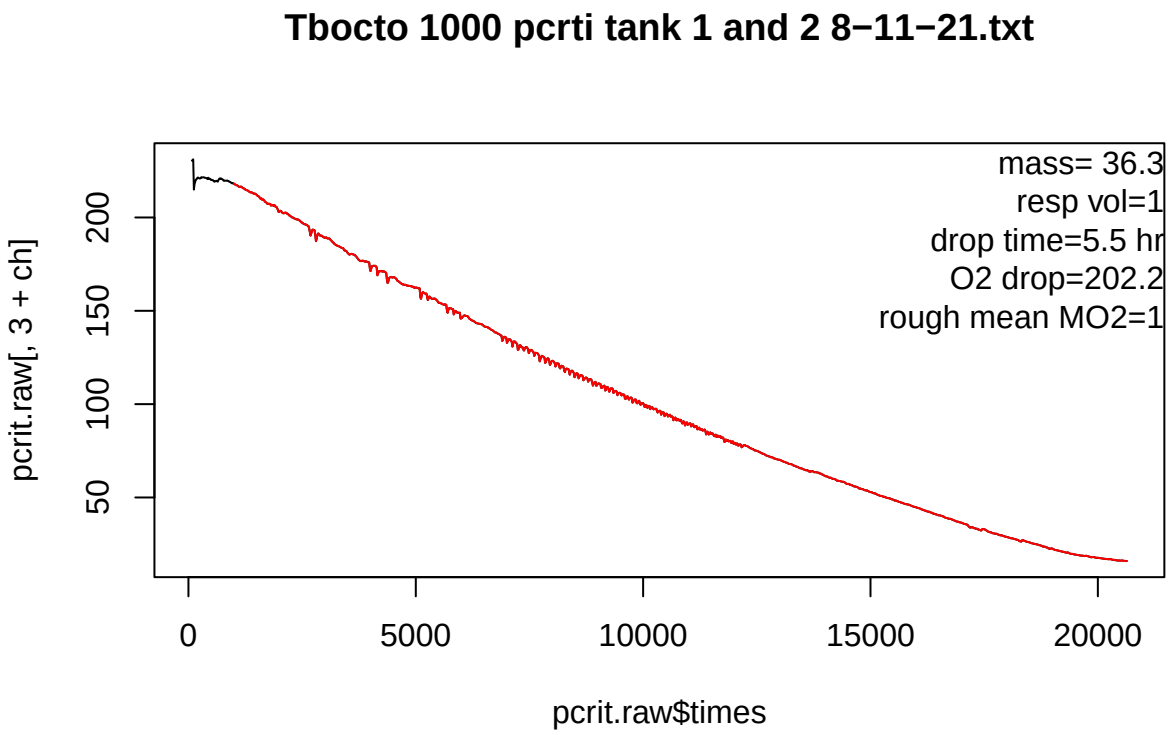

Alpha @ MR of 6.15 = 6.337
Breakpoint = 15.447
LLO @ MR of 6.15 = 10.509
NLR (Hyperbola) = 24.757
Sub−PI = 15.08
DROPPED FROM ANALYSIS
pO2 did not get low enough
Effect of po2
0
5
10
15
8
10
12
14
16
18
po2

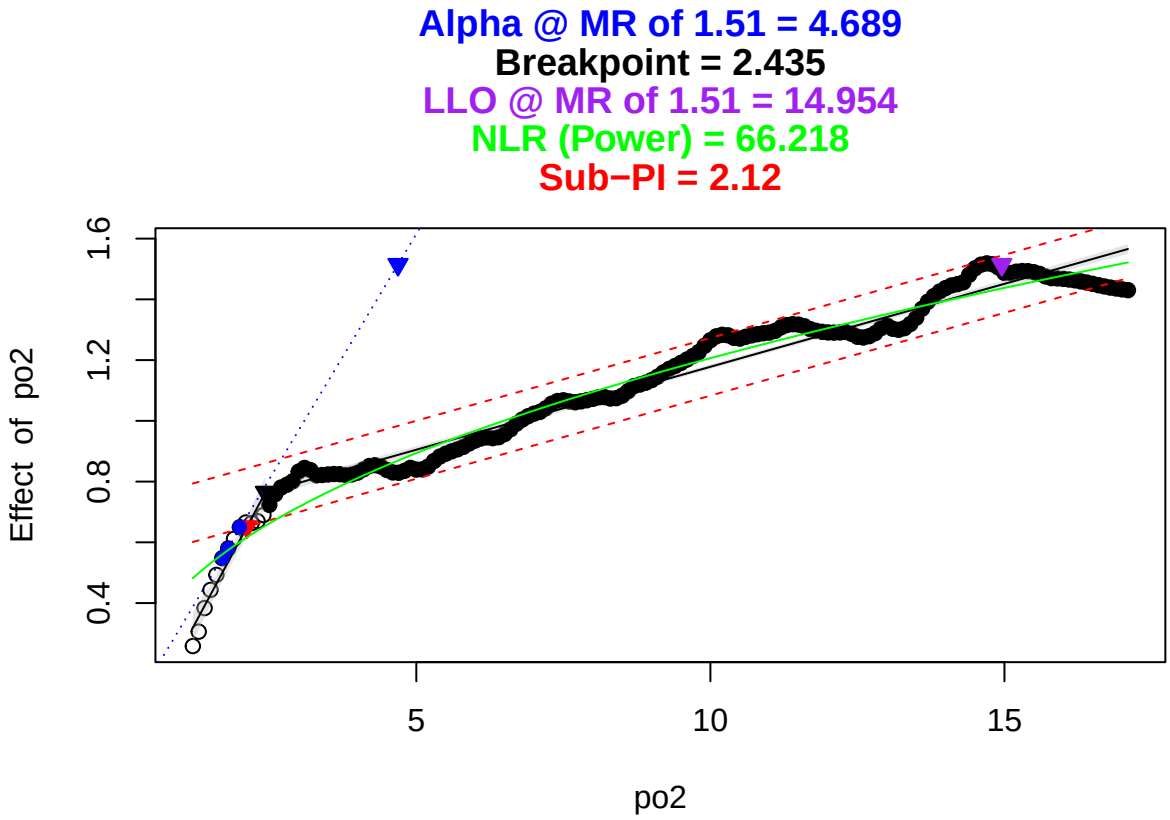

tbocto 1000 pcrit tank 3 and 4 day 7 8−19−21−ch1.txt
mass= 13
resp vol=1
drop time=7.9 hr
O2 drop=194.1
rough mean MO2=1.9
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
25000
pcrit.raw$times

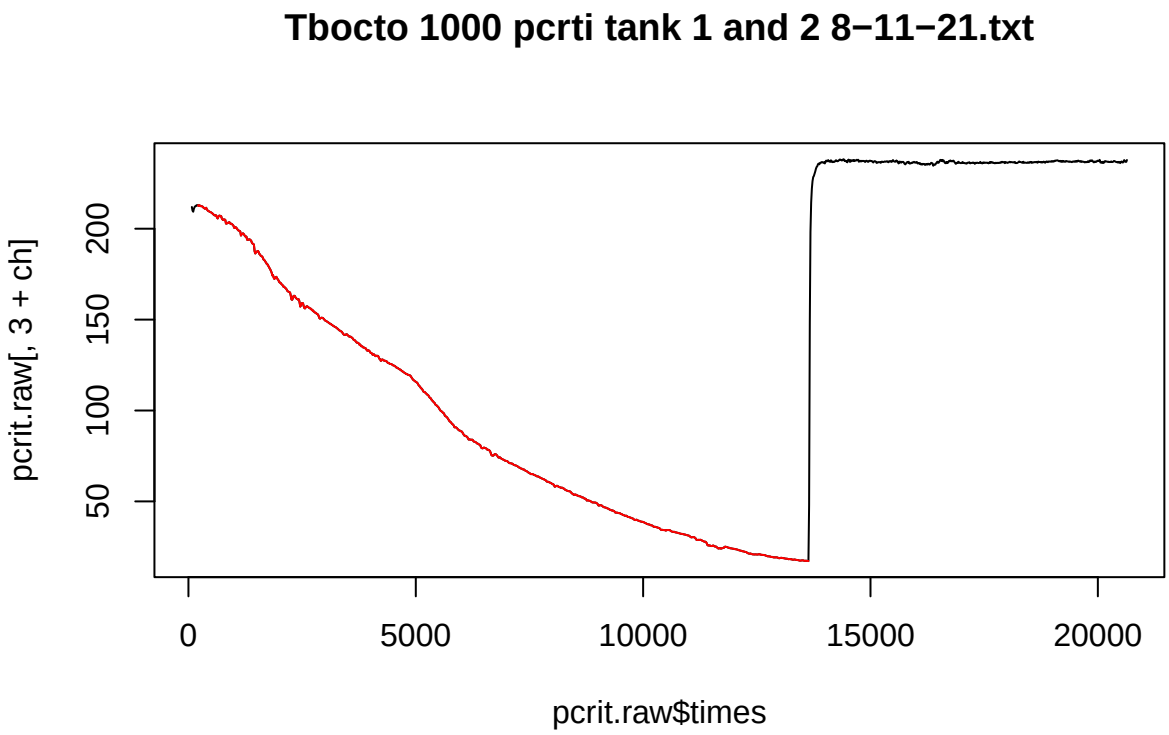

Alpha @ MR of 3.21 = 8.306
Breakpoint = 9
LLO @ MR of 3.21 = 9.657
NLR (Pareto) = 6.088
Sub−PI = 6.81
Effect of po2
0.5
1.5
2.5
3.5
5
10
15
po2

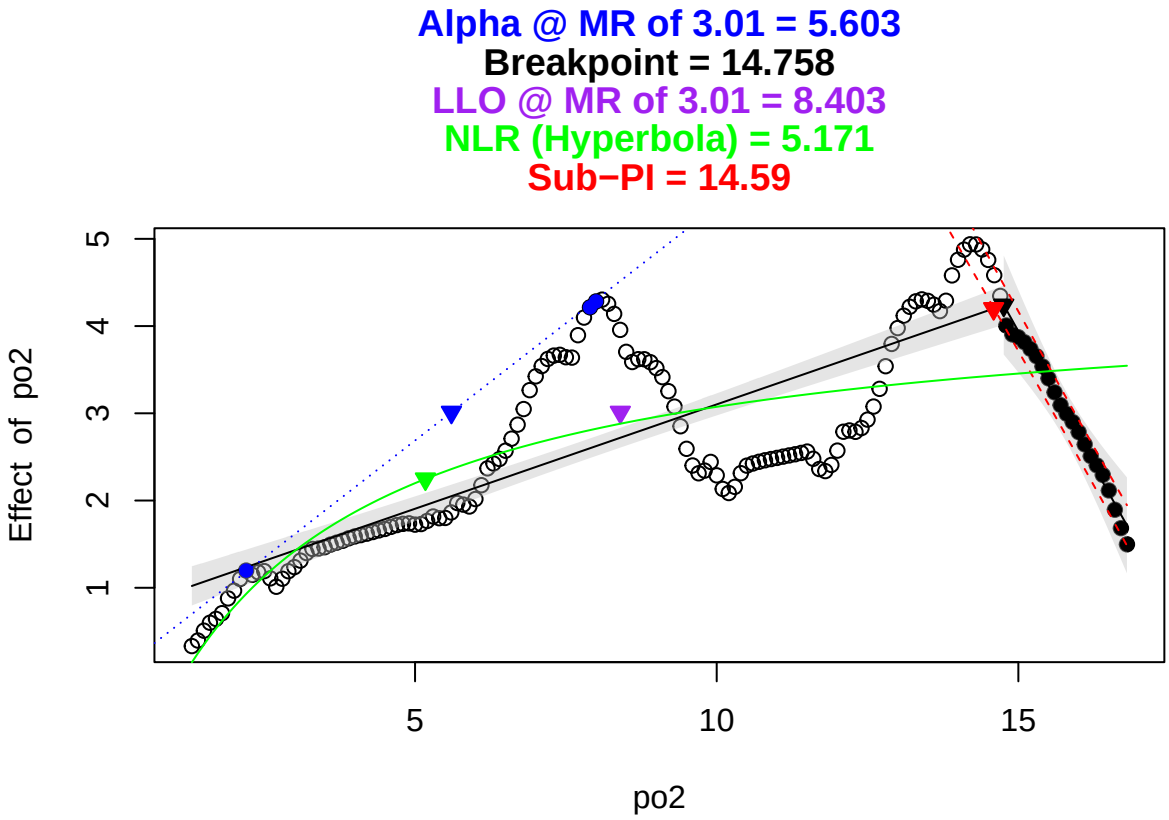

tbocto 1000 pcrit tank 3 and 4 day 7 8−19−21−ch1.txt
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
20000
25000
pcrit.raw$times

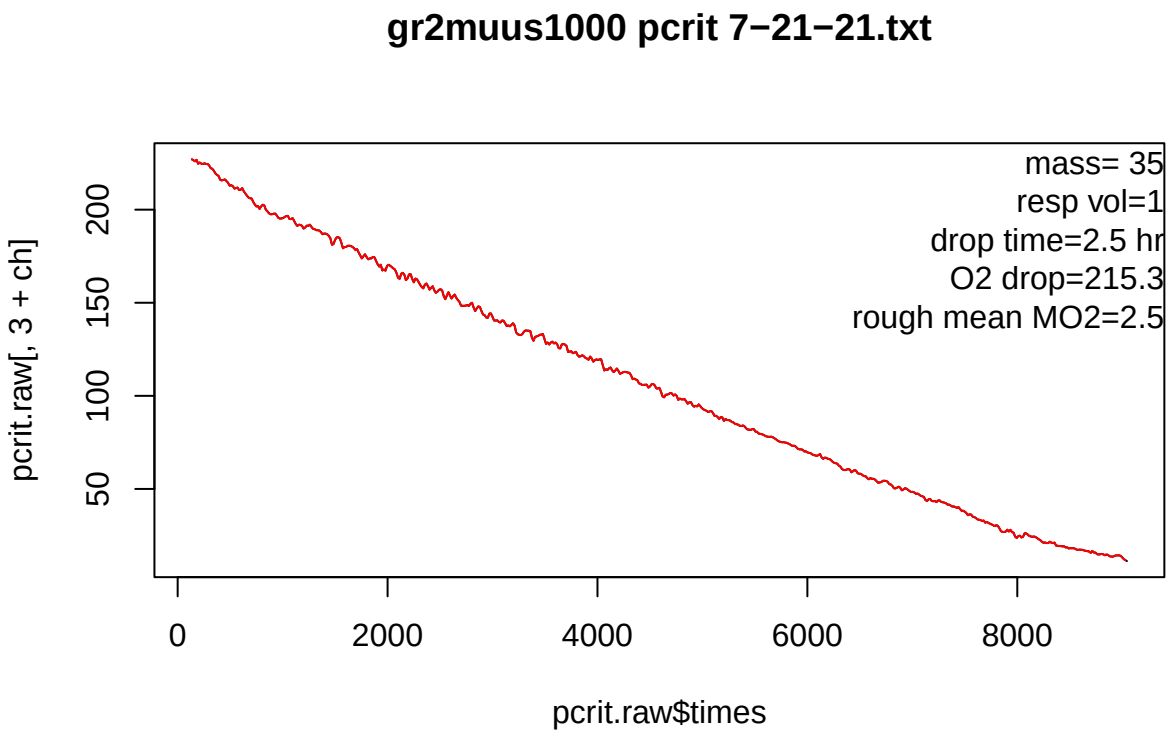

Alpha @ MR of 6.15 = 4.788
Breakpoint = 13.7
LLO @ MR of 6.15 = 7.338
NLR (Hyperbola) = 11.118
Sub−PI = 13.45
Effect of po2
0
5
10
15
5
10
15
po2

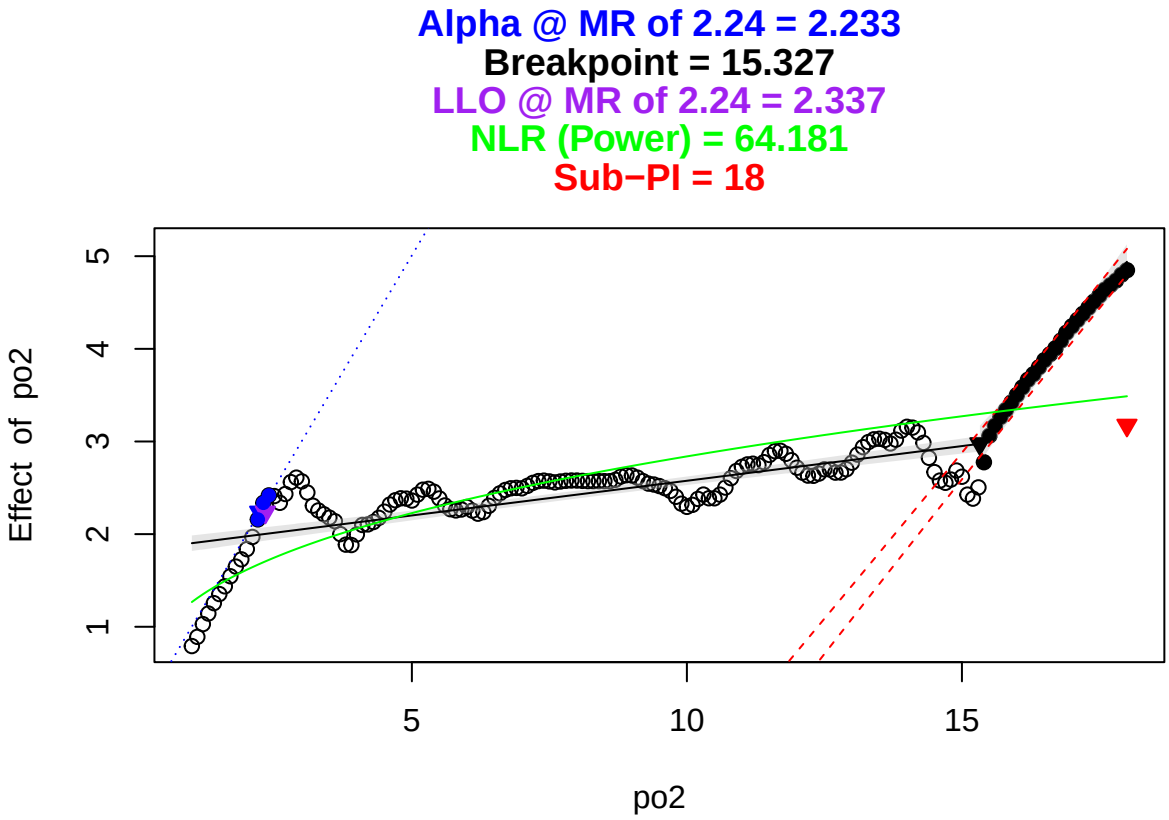

Tbocto 1000 pcrti tank 1 and 2 8−11−21.txt
mass= 36.3
resp vol=1
drop time=5.5 hr
O2 drop=202.2
rough mean MO2=1
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
pcrit.raw$times

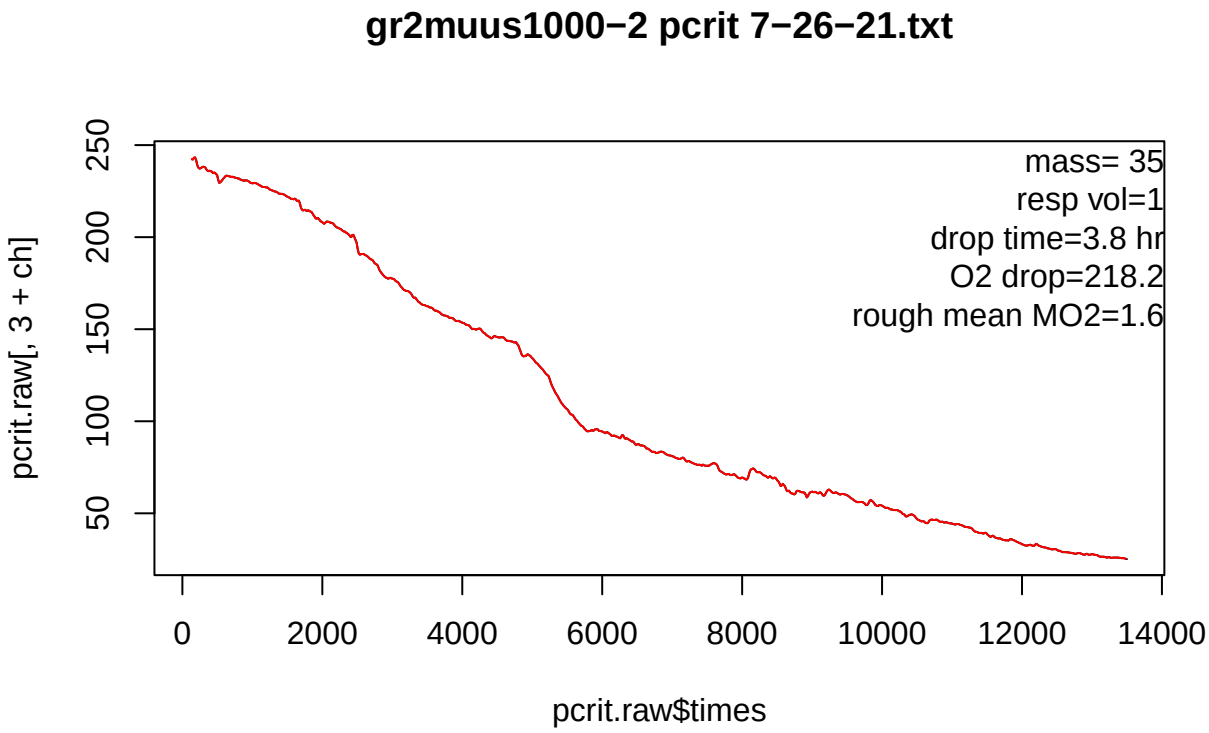

Alpha @ MR of 1.51 = 4.689
Breakpoint = 2.435
LLO @ MR of 1.51 = 14.954
NLR (Power) = 66.218
Sub−PI = 2.12
Effect of po2
0.4
0.8
1.2
1.6
5
10
15
po2

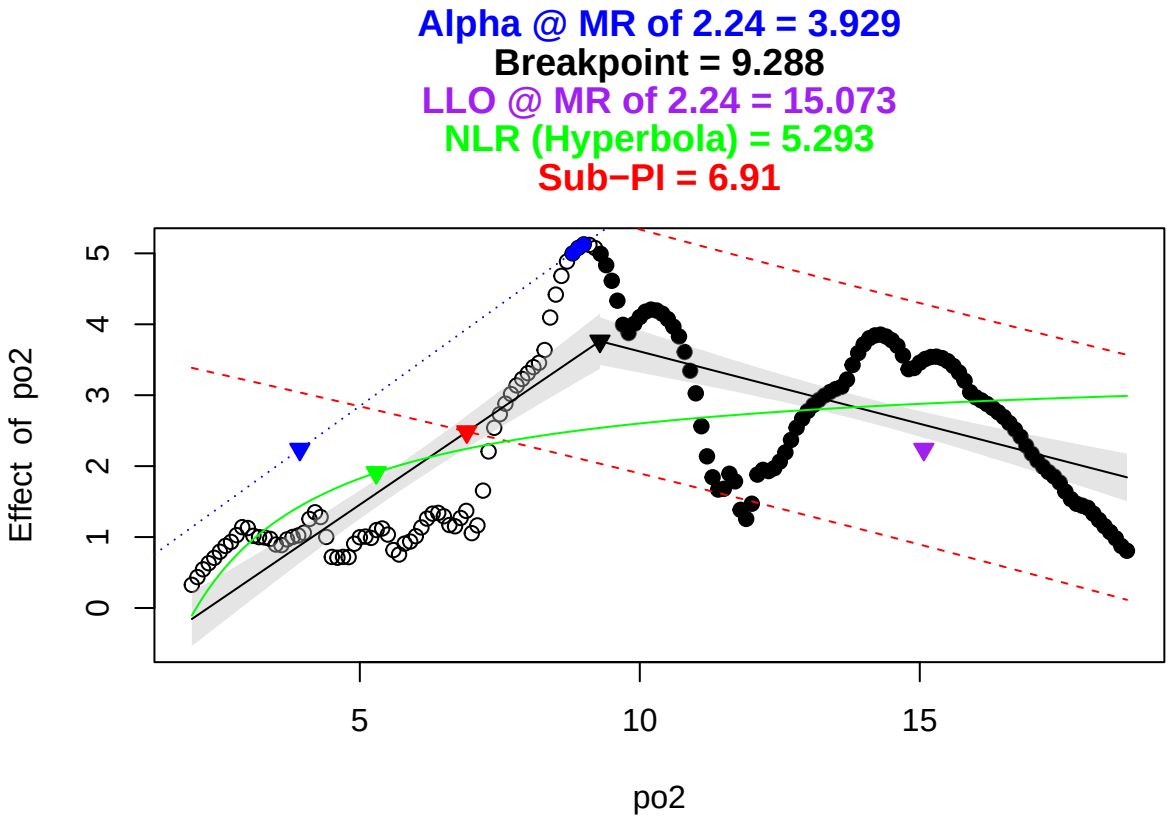

Tbocto 1000 pcrti tank 1 and 2 8−11−21.txt
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
pcrit.raw$times

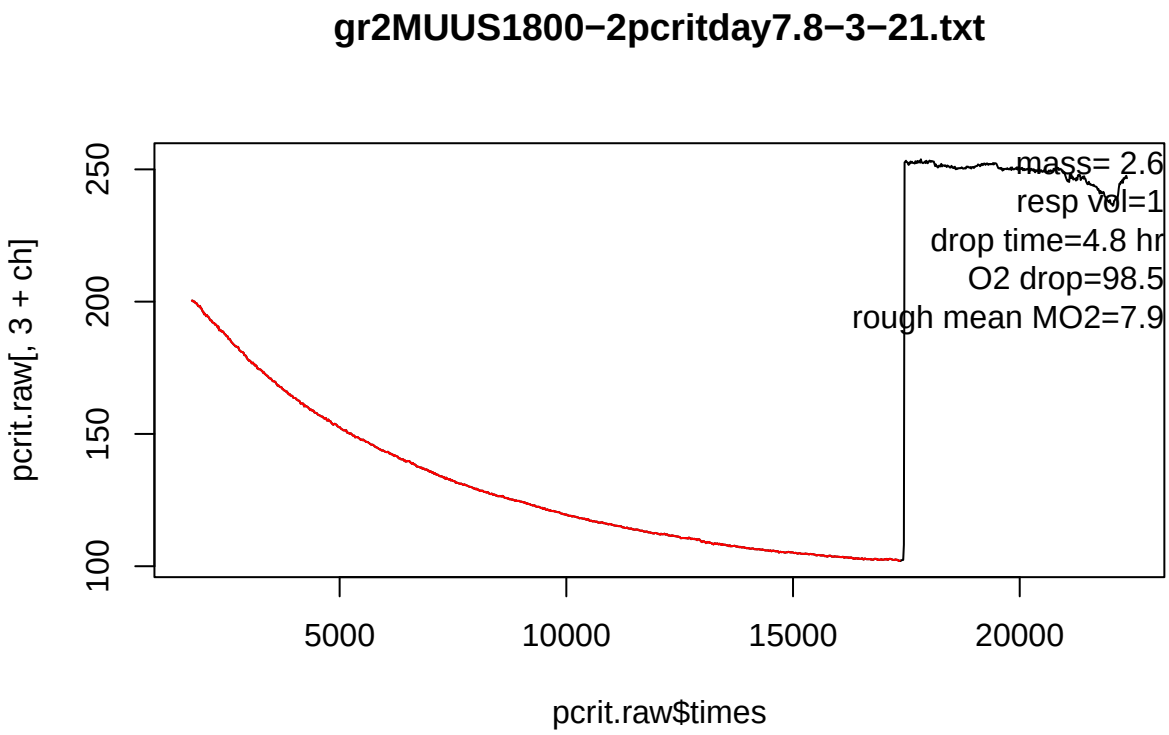

Alpha @ MR of 3.01 = 5.603
Breakpoint = 14.758
LLO @ MR of 3.01 = 8.403
NLR (Hyperbola) = 5.171
Sub−PI = 14.59
Effect of po2
1
2
3
4
5
5
10
15
po2

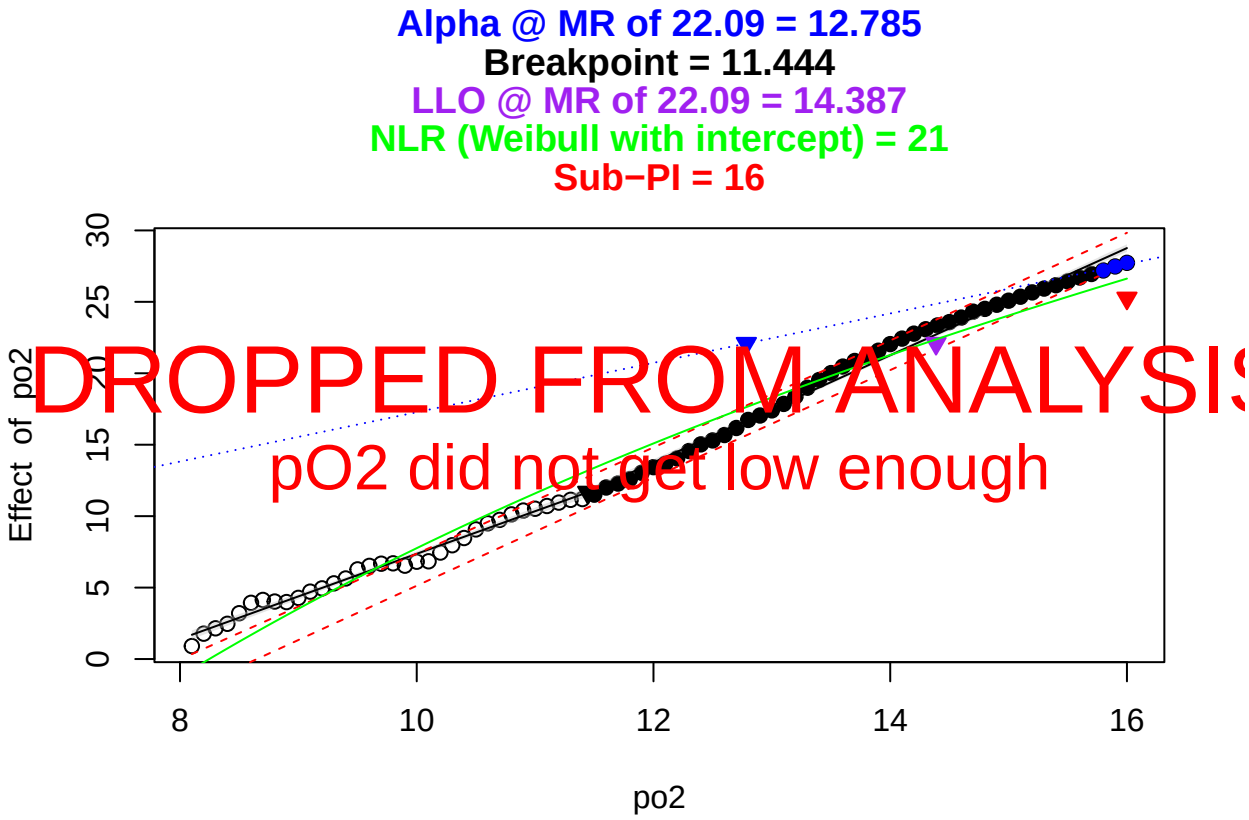

gr2muus1000 pcrit 7−21−21.txt
mass= 35
resp vol=1
drop time=2.5 hr
O2 drop=215.3
rough mean MO2=2.5
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
pcrit.raw$times

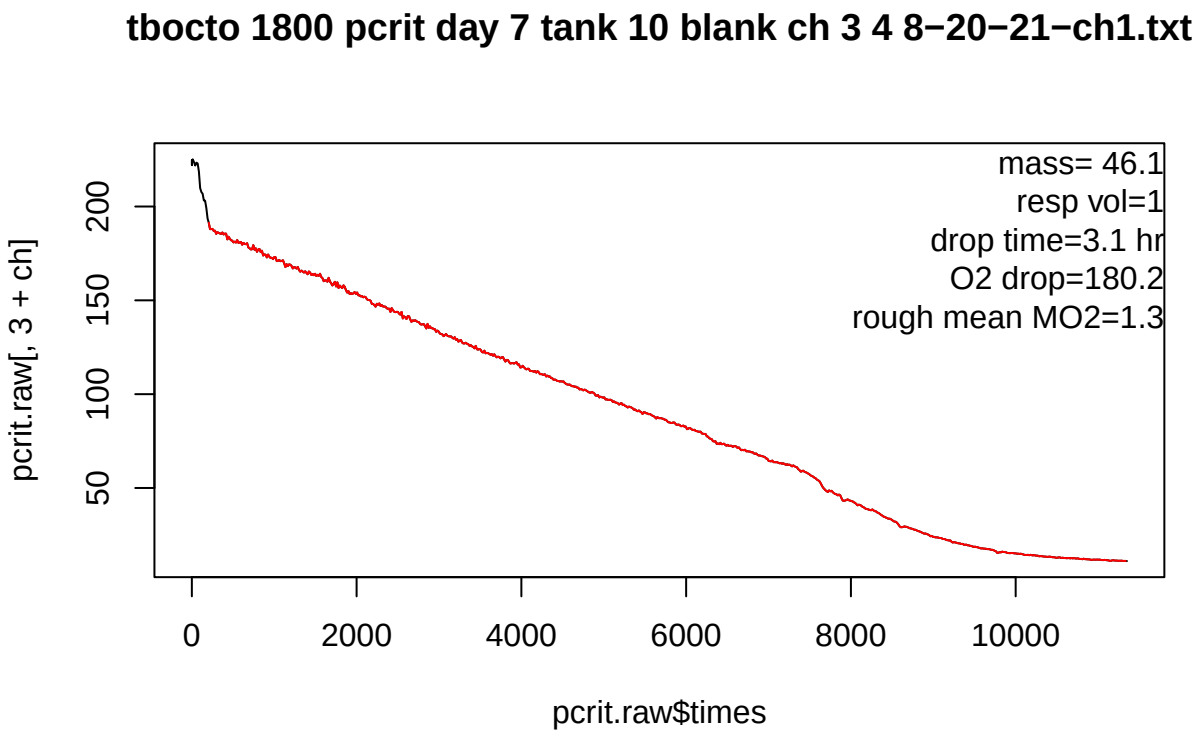

Alpha @ MR of 2.24 = 2.233
Breakpoint = 15.327
LLO @ MR of 2.24 = 2.337
NLR (Power) = 64.181
Sub−PI = 18
Effect of po2
1
2
3
4
5
5
10
15
po2

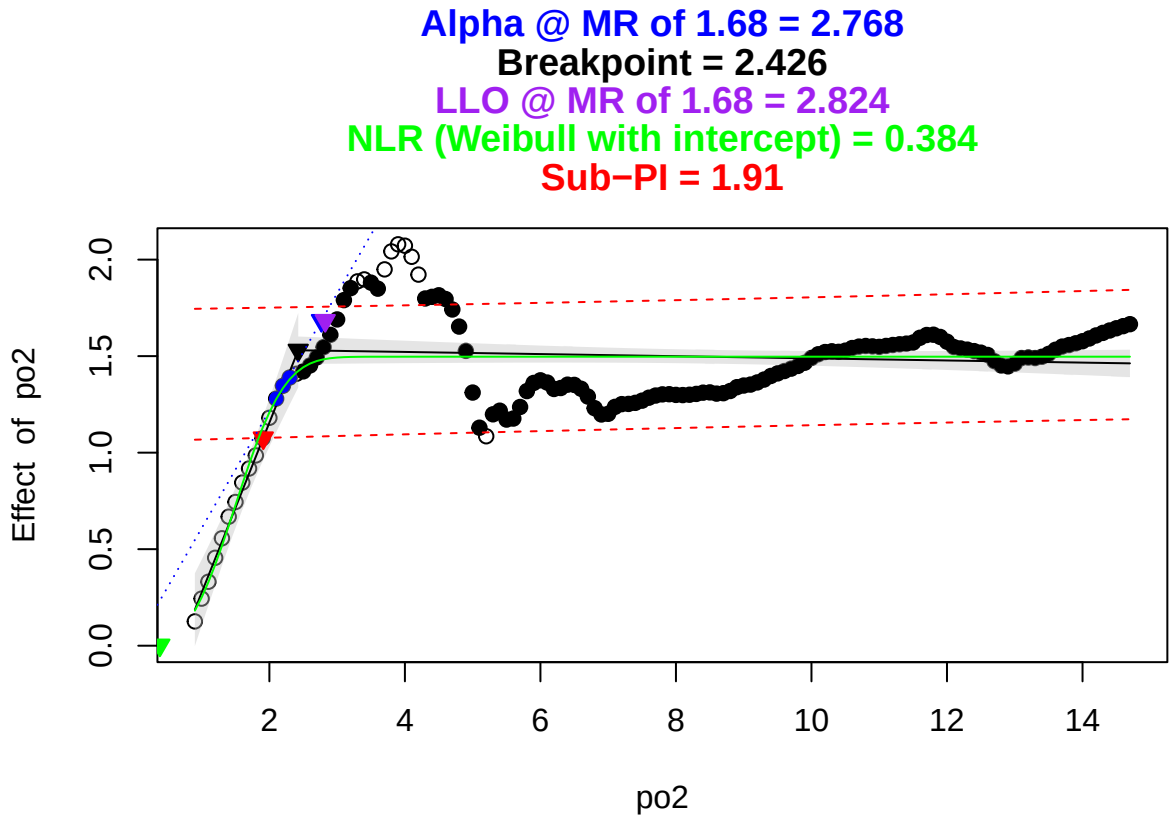

gr2muus1000−2 pcrit 7−26−21.txt
mass= 35
resp vol=1
drop time=3.8 hr
O2 drop=218.2
rough mean MO2=1.6
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
2000
4000
6000
8000
10000
12000
14000
pcrit.raw$times


Alpha @ MR of 2.24 = 3.929
Breakpoint = 9.288
LLO @ MR of 2.24 = 15.073
NLR (Hyperbola) = 5.293
Sub−PI = 6.91
Effect of po2
0
1
2
3
4
5
5
10
15
po2


gr2MUUS1800−2pcritday7.8−3−21.txt
mass= 2.6
resp vol=1
drop time=4.8 hr
O2 drop=98.5
rough mean MO2=7.9
pcrit.raw[, 3 + ch]
100
150
200
250
5000
10000
15000
20000
pcrit.raw$times


Alpha @ MR of 22.09 = 12.785
Breakpoint = 11.444
LLO @ MR of 22.09 = 14.387
NLR (Weibull with intercept) = 21
Sub−PI = 16
DROPPED FROM ANALYSIS
pO2 did not get low enough
Effect of po2
0
5
10
15
20
25
30
8
10
12
14
16
po2

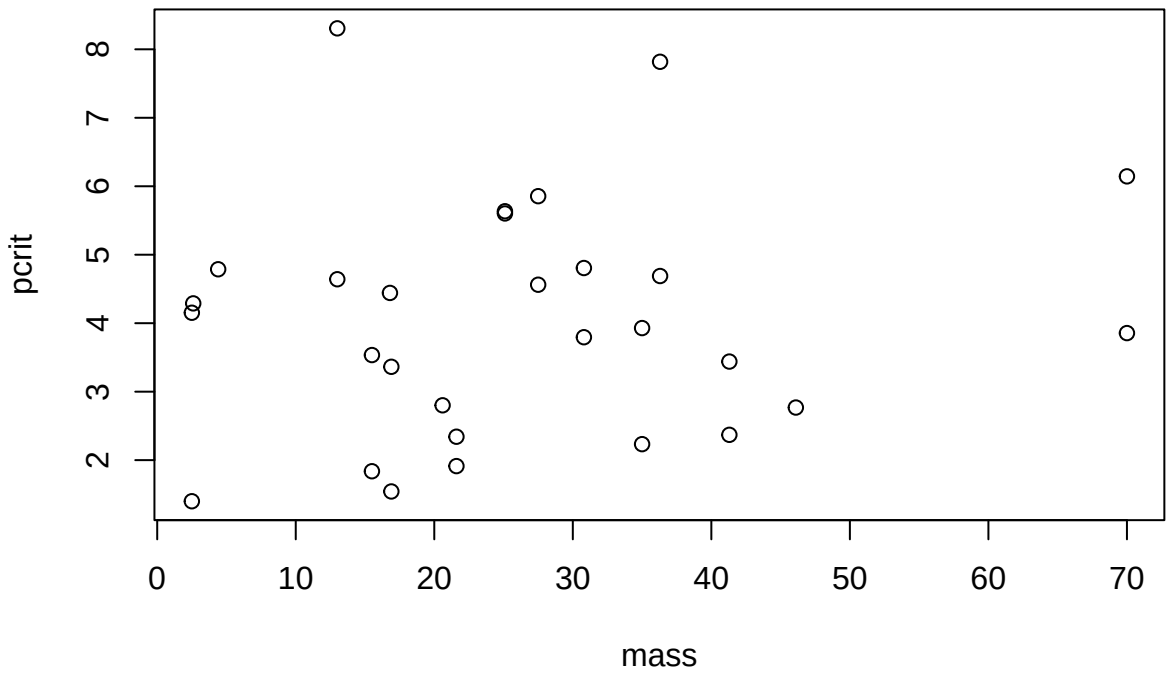

tbocto 1800 pcrit day 7 tank 10 blank ch 3 4 8−20−21−ch1.txt
mass= 46.1
resp vol=1
drop time=3.1 hr
O2 drop=180.2
rough mean MO2=1.3
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
10000
pcrit.raw$times



Next, I remove the measurements for which the data was excluded (runs did not reach oxygen level of 50 $mmHgO_2$). Then I remove the file for which there is no initial data.

```
pcrits=pcrits[complete.cases(pcrits),]
pcrits=pcrits[pcrits$filename!="GR1 Muus1000 pcrit 7-21-21.txt",]
```

```
plot(pcrit~mass,data=pcrits)
```



Complete table of final pcrit and alpha values

```
pcrits.kable=pcrits[,c(3:6,8:9)]
#pcrits.kable$rmr=round(pcrits.kable$rmr,2)
pcrits.kable$pcrit=round(pcrits.kable$pcrit,2)
pcrits.kable$alpha=round(pcrits.kable$alpha,2)
colnames(pcrits.kable)=c("Octopus ID",
                         "mass (g)",
                         "pCO~2~ ($\\mu$atm)",
                         "day",
#                          "Routine Metabolic Rate ($\\mu$O~2~ g^-1^ hr^-1^)",
                         "P~CRIT~ (kPa)",
                         "Oxygen Supply Capacity ($\\alpha$)")
pcrits.kable=pcrits.kable[order(pcrits.kable$day),]
pcrits.kable=pcrits.kable[order(pcrits.kable$`Octopus ID`),]
kable(pcrits.kable,align=c("l",rep("c",4),"r"),row.names = F)
```

Octopus ID	mass (g)	pCO_2 (μatm)	day	P_{CRIT} (kPa)	Oxygen Supply Capacity (α)
1-1	30.8	1800	1	3.80	0.66
1-1	30.8	1800	7	4.81	0.52
1-2	20.6	1000	1	2.80	0.79
1-3	2.5	1800	1	1.40	1.64
1-3	2.5	1800	7	4.15	0.55
2-1	16.8	1800	7	4.44	0.33

Octopus ID	mass (g)	pCO_2 (μatm)	day	P_{CRIT} (kPa)	Oxygen Supply Capacity (α)
2-2	35.0	1000	1	2.23	1.00
2-2	35.0	1000	7	3.93	0.57
2-3	2.6	1800	1	4.29	0.85
3-1	70.0	1800	1	6.14	0.24
3-1	70.0	1800	7	3.86	0.48
3-2	21.6	1000	1	1.91	1.14
3-2	21.6	1000	7	2.34	0.93
3-3	16.9	1800	1	1.54	1.40
3-3	16.9	1800	7	3.36	0.64
4-1	27.5	1800	1	4.56	0.50
4-1	27.5	1800	7	5.85	0.39
4-2	15.5	1000	1	1.84	1.37
4-2	15.5	1000	7	3.53	0.71
4-3	41.3	1800	1	2.37	0.89
4-3	41.3	1800	7	3.44	0.62
5-1	36.3	1000	1	4.69	0.32
5-1	36.3	1000	7	7.82	0.19
5-2	25.1	1000	1	5.60	0.54
5-2	25.1	1000	7	5.63	0.43
5-3	13.0	1000	1	4.64	0.69
5-3	13.0	1000	7	8.31	0.39
5-4	4.4	1000	7	4.79	1.28
5-5	46.1	1800	1	2.77	0.61

5 P_{CRIT} Linear mixed effect model

5.1 setting pCO2 to factor class:

```
pcrits$pco2=as.factor(pcrits$pco2)
```

5.2 Next I set orthogonal contrasts:

```
contrasts(pcrits$pco2)=contr.poly(2)
```

5.3 Running the linear mixed effects model and ANOVA using type III sum of squares:

```
pcrits.lme=lme(pcrit~mass+pco2+day,random=~1|octo,
               correlation=corAR1(form=~day|octo),
                data=pcrits,na.action=na.omit)

Anova(pcrits.lme,type="III")
```

```
## Analysis of Deviance Table (Type III tests)
##
## Response: pcrit
##               Chisq Df Pr(>Chisq)
## (Intercept) 14.8709  1  0.0001151 ***
## mass         0.4563  1  0.4993495
## pco2         0.4646  1  0.4954673
## day         10.5386  1  0.0011691 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

5.4 Summary of LME

```
pcrit.em=data.frame(emmeans(pcrits.lme,~pco2+day+mass))
```

```
## Warning: contrasts dropped from factor pco2
```

```
pcrit.em
```

```
##   pco2 day     mass   emmean        SE df lower.CL upper.CL
## 1 1000   1 26.25862 3.635840 0.5676006 11 2.386560 4.885121
## 2 1800   1 26.25862 3.135719 0.5341027 11 1.960167 4.311271
## 3 1000   7 26.25862 4.998403 0.5699236 11 3.744010 6.252797
## 4 1800   7 26.25862 4.498282 0.5479037 11 3.292354 5.704210
```

```
pcrit.df=
data.frame(cbind(
  as.numeric(as.character(pcrit.em$pco2)),
  pcrit.em$day,
  sprintf("%.2f",signif(pcrit.em$emmean,3)),
  paste(sprintf("%.2f",signif(data.frame(pcrit.em)$lower.CL,3)),
        "-",
        sprintf("%.2f",signif(data.frame(pcrit.em)$upper.CL,3)))
))
pcrit.df=pcrit.df[order(pcrit.df[,1]),]
pcrit.df
```

```
##     X1 X2   X3          X4
## 1 1000  1 3.64 2.39 - 4.89
## 3 1000  7 5.00 3.74 - 6.25
## 2 1800  1 3.14 1.96 - 4.31
## 4 1800  7 4.50 3.29 - 5.70
```

```
colnames(pcrit.df)=c("pCO~2~ ($\\mu$atm)",
                     "day",
                     "P~CRIT~ (kPa)",
                     "P~CRIT~ 95% CI")
kable(pcrit.df,align="c",row.names = F)
```

pCO_2 (μatm)	day	P_{CRIT} (kPa)	P_{CRIT} 95% CI
1000	1	3.64	2.39 - 4.89
1000	7	5.00	3.74 - 6.25
1800	1	3.14	1.96 - 4.31
1800	7	4.50	3.29 - 5.70

6 Plotting the Critical Oxygen Pressure results

Assigning the colors for the treatments.

```
hi.co2.col="#790000ff"
lo.co2.col="#838fd5ff"
```

Next, making the figures in SVG format.

```
svg(filename = "Figure_4.svg",width=3.5,height=3.5,pointsize=6)
par(fig=c(0.04,1,0,1))
boxplot(pcrit~pco2+day,data=pcrits,range=0,
        axes=F,col=c(lo.co2.col,hi.co2.col,lo.co2.col,hi.co2.col),ylab="",xlab="")
box(lwd=2)
abline(v=2.5)
axis(1, at=c(1.5,3.5),labels = c("Day 1","Day 7"),tick=F,cex.axis=1.5)
axis(2,at=c(2,4,6,8),cex.axis=1.5)
mtext(expression("Critical Oxygen Pressure (kPa)"),side=2,cex=1.8,line=3)
legend(1,8,pt.bg=c(lo.co2.col,hi.co2.col),legend=c("1000","1800"),
       pch=22,cex=1.3,pt.cex=3.2,bty="n",adj=c(0,0.5),title=expression("pCO"[2]*" ("*mu*"atm)"))
dev.off()
```

```
## pdf
##   2
```

Converting the image to a png and jpg to be displayed in the RMarkdown.

```
cairosvg Figure_4.svg -o Figure_4.png -d 300
convert Figure_4.png Figure_4.jpg
```

Converting to eps for submission.

```
inkscape Figure_4.svg -o Figure_4.eps --export-ignore-filters --export-ps-level=3
```

7 Oxygen supply capacity (α) linear mixed effect model

7.1 Running the linear mixed effects model and ANOVA using type III sum of squares:

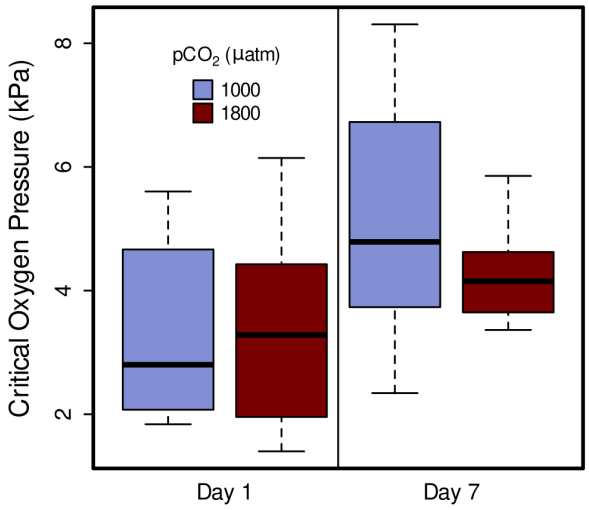


Figure 1: Critical oxygen pressure of Muusoctopus leioderma at differing pCO2 treatments

```
alpha.lme=lme(alpha~mass*pco2*day,random=~1|octo,
              correlation=corAR1(form=~day|octo),
               data=pcrits,na.action=na.omit)

alpha.anova=Anova(alpha.lme,type="III")
```

```
options(scipen=10)
alpha.lme.table=cbind(
  c("Mass","pCO~2~","Day"),
  round(alpha.anova$Chisq[2:4],2),
  alpha.anova$Df[2:4],
  round(alpha.anova$`Pr(>Chisq)`[2:4],6)
)


colnames(alpha.lme.table)=c("Factor","Chi-square", "DF", "p-value")

kable(alpha.lme.table)
```

Factor	Chi-square	DF	p-value
Mass	9.84	1	0.00171
pCO_2	0.06	1	0.808008
Day	17.35	1	0.000031

7.2 Summary of LME

```
alpha.em=data.frame(emmeans(alpha.lme,~pco2+day+mass))
```

```
## Warning: contrasts dropped from factor pco2
```

```
alpha.em
```

```
##   pco2 day     mass    emmean        SE df  lower.CL  upper.CL
## 1 1000   1 26.25862 0.8040022 0.1151260  8 0.5385212 1.0694832
## 2 1800   1 26.25862 0.8925850 0.1019162  8 0.6575658 1.1276041
## 3 1000   7 26.25862 0.5266816 0.1181234  8 0.2542887 0.7990746
## 4 1800   7 26.25862 0.4591574 0.1058851  8 0.2149860 0.7033289
```

```
alpha.df=
data.frame(cbind(
  as.numeric(as.character(alpha.em$pco2)),
  alpha.em$day,
  sprintf("%.2f",signif(alpha.em$emmean,3)),
  paste(sprintf("%.2f",signif(data.frame(alpha.em)$lower.CL,3)),
        "-",
        sprintf("%.2f",signif(data.frame(alpha.em)$upper.CL,3)))
))
alpha.df=alpha.df[order(alpha.df[,1]),]
alpha.df
```

```
##     X1 X2   X3          X4
## 1 1000  1 0.80 0.54 - 1.07
## 3 1000  7 0.53 0.25 - 0.80
## 2 1800  1 0.89 0.66 - 1.13
## 4 1800  7 0.46 0.21 - 0.70
```

```
colnames(alpha.df)=c("pCO~2~ ($\\mu$atm)",
                     "day",
                     "Oxygen Supply Capacity ($\\alpha$)",
                     "Oxygen Supply Capacity 95% CI")
kable(alpha.df,align="c",row.names = F)
```

pCO_2 (μatm)	day	Oxygen Supply Capacity (α)	Oxygen Supply Capacity 95% CI
1000	1	0.80	0.54 - 1.07
1000	7	0.53	0.25 - 0.80
1800	1	0.89	0.66 - 1.13
1800	7	0.46	0.21 - 0.70

8 Oxygen Supply Capacity figure

8.1 Predicted values

First I am getting the model predicted values for each treatment between the max an minimum mass values.

```
seq1.1800=seq(from=min(pcrits$mass[pcrits$pco2==1800]),
              to=max(pcrits$mass[pcrits$pco2==1800]),
              length.out=100)

df1.1800=data.frame(
  day=rep(1,100),
  mass=seq1.1800,
  pco2=as.factor(rep(1800,100))
)
pred1.1800= predict(alpha.lme,newdata = df1.1800,level=0)

seq1.1000=seq(from=min(pcrits$mass[pcrits$pco2==1000]),
              to=max(pcrits$mass[pcrits$pco2==1000]),
              length.out=100)

df1.1000=data.frame(
  day=rep(1,100),
  mass=seq1.1000,
  pco2=as.factor(rep(1000,100))
)

pred1.1000=predict(alpha.lme,newdata = df1.1000,level=0)

seq7.1800=seq(from=min(pcrits$mass[pcrits$pco2==1800]),
              to=max(pcrits$mass[pcrits$pco2==1800]),
              length.out=100)

df7.1800=data.frame(
  day=rep(7,100),
  mass=seq7.1800,
  pco2=as.factor(rep(1800,100))
)

pred7.1800=predict(alpha.lme,newdata = df7.1800,level=0)

seq7.1000=seq(from=min(pcrits$mass[pcrits$pco2==1000]),
              to=max(pcrits$mass[pcrits$pco2==1000]),
              length.out=100)

df7.1000=data.frame(
  day=rep(7,100),
  mass=seq7.1000,
  pco2=as.factor(rep(1000,100))
)

pred7.1000=predict(alpha.lme,newdata = df7.1000,level=0)
```

Assigning the colors for the treatments.

```
hi.co2.col="#790000ff"
lo.co2.col="#838fd5ff"
```

Next, I am actually plotting it.

```
svg(filename="Figure_5.svg",height=3.5,width=3.5,pointsize=6)
par(fig=c(0.04,1,0,1))
plot(alpha~mass,data=pcrits,axes=F,ylab="",xlab="",type="n")
box(lwd=2)
axis(1,lwd=2,cex.axis=2)
axis(2,lwd=2,cex.axis=1.5)
mtext(expression("Oxygen Supply Capacity ("*mu*"molO"[2]*" g"^-1*"hr"^-1*"kPa"^-1*")"),
      side=2,cex=1.8,line=2.5)
mtext("Mass (g)",side=1,cex=1.8,line=2.5)

points(alpha~mass,data=pcrits[pcrits$pco2==1000&pcrits$day==1,],
       pch=22,bg="white",col=lo.co2.col,cex=1.5)
points(alpha~mass,data=pcrits[pcrits$pco2==1000&pcrits$day==7,],
       pch=22,bg=lo.co2.col,cex=1.5)
points(alpha~mass,data=pcrits[pcrits$pco2==1800&pcrits$day==7,],
       pch=21,bg=hi.co2.col,cex=1.5)
points(alpha~mass,data=pcrits[pcrits$pco2==1800&pcrits$day==1,],
       pch=21,bg="white",col=hi.co2.col,cex=1.5)
lines(seq1.1800,pred1.1800,col=hi.co2.col,lwd=2,lty=2)
lines(seq1.1000,pred1.1000,col=lo.co2.col,lwd=2,lty=2)
lines(seq7.1800,pred7.1800,col=hi.co2.col,lwd=2,lty=1)
lines(seq7.1000,pred7.1000,col=lo.co2.col,lwd=2,lty=1)
legend("topright", c(expression("1000 "*mu*"atm pCO"["2"]*", day 1"),
                     expression("1000 "*mu*"atm pCO"["2"]*", day 7"),
                     expression("1800 "*mu*"atm pCO"["2"]*", day 1"),
                     expression("1800 "*mu*"atm pCO"["2"]*", day 7")),
       pch = c(22,22,21,21),bty="n",title = expression("Treatment pCO"["2"]),
       pt.bg=c("white",lo.co2.col,"white",hi.co2.col),col=c(lo.co2.col,"black",hi.co2.col,"black"),
       inset = .02,cex=1.3,box.lwd=2,pt.lwd=1,pt.cex=2)

dev.off()
```

```
## pdf
##   2
```

Converting the image to a png and jpg to be displayed in the RMarkdown.

```
cairosvg Figure_5.svg -o Figure_5.png -d 300
convert Figure_5.png Figure_5.jpg
```

Converting to eps for submission.

```
inkscape Figure_5.svg -o Figure_5.eps --export-ignore-filters --export-ps-level=3
```

9 Finding the slopes of the treatments

Here I am producing a table of the slope and intercepts of the linear mixed effects model of routine metabolic rates. These values correspond to the logged mass and logged RMR, and do not translate to the untransformed data. The relationship between the untransformed data is not linear, and therefore has no slope. Also, because the log of 0 is infinite, these intercepts correspond instead to a mass of 1g (exp(0)=1).

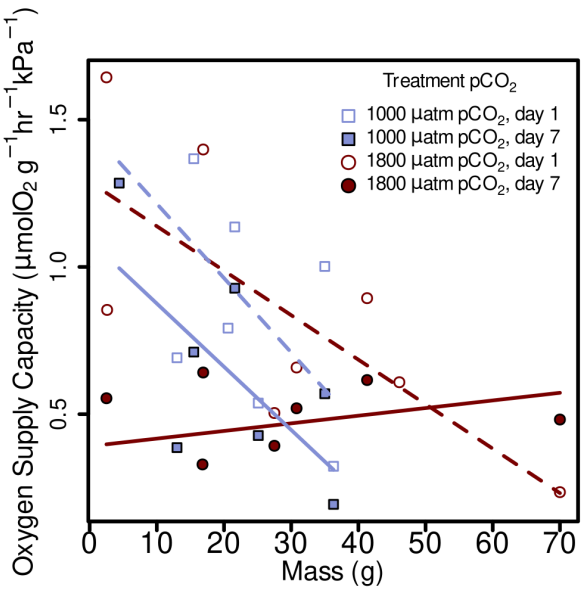


Figure 2: Oxygen supply capacity from M.leioderma in Burrows Bay, Anacortes Washington

```
mass=0
alpha.slope=data.frame(pCO2=c(1000,1000,1800,1800),
           Day=c(1,7,1,7),
           Intercept=round(predict(alpha.lme,
                  newdata=data.frame(
                       day=c(1,7,1,7),
                       mass=rep(mass,4),
                       pco2=as.factor(c(1000,1000,1800,1800))),
                     level=0)
                [1:4],2),
           Slope=c(
            round(-1*(pred1.1000[1]-pred1.1000[length(pred1.1000)])/diff(range(seq1.1000)),3),
            round(-1*(pred7.1000[1]-pred7.1000[length(pred7.1000)])/diff(range(seq7.1000)),3),
            round(-1*(pred1.1800[1]-pred1.1800[length(pred1.1800)])/diff(range(seq1.1800)),3),
            round(-1*(pred7.1800[1]-pred7.1800[length(pred7.1800)])/diff(range(seq7.1800)),3)
            )
           )

colnames(alpha.slope)[1]="pCO~2~ ($\\mu$atm)"
kable(alpha.slope,align="c",row.names=F)
```

pCO_2 (μatm)	Day	Intercept	Slope
1000	1	1.47	-0.025
1000	7	1.09	-0.021
1800	1	1.29	-0.015
1800	7	0.39	0.003

10 Is there a relationship between RMR and alpha?

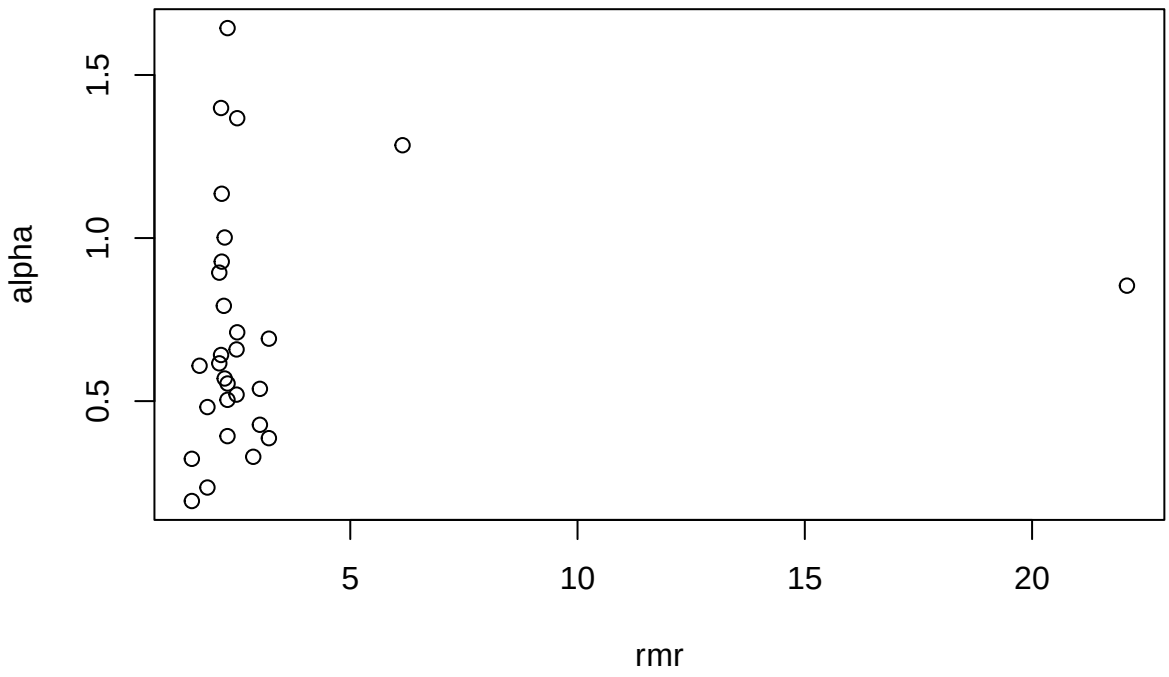
One reviewer asked if alpha was correlated with mass or with RMR. THe previous analysis answers the first part of that question. Here I attempt to answer the second. I did not include RMR in the linear mixed effects model because there is a well-document relationship between RMR and mass, as can be seen in Figure 3 of this analysis, and I did not want to include a

```
summary(aov(lm(alpha~rmr,data=pcrits)))
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## rmr          1  0.067 0.06668   0.484  0.493
## Residuals   27  3.720 0.13778
```

With a p-value of 0.493, there appears to be no significant relationship between alpha and rmr. Here is the graph.

```
plot(alpha~rmr,data=pcrits)
```



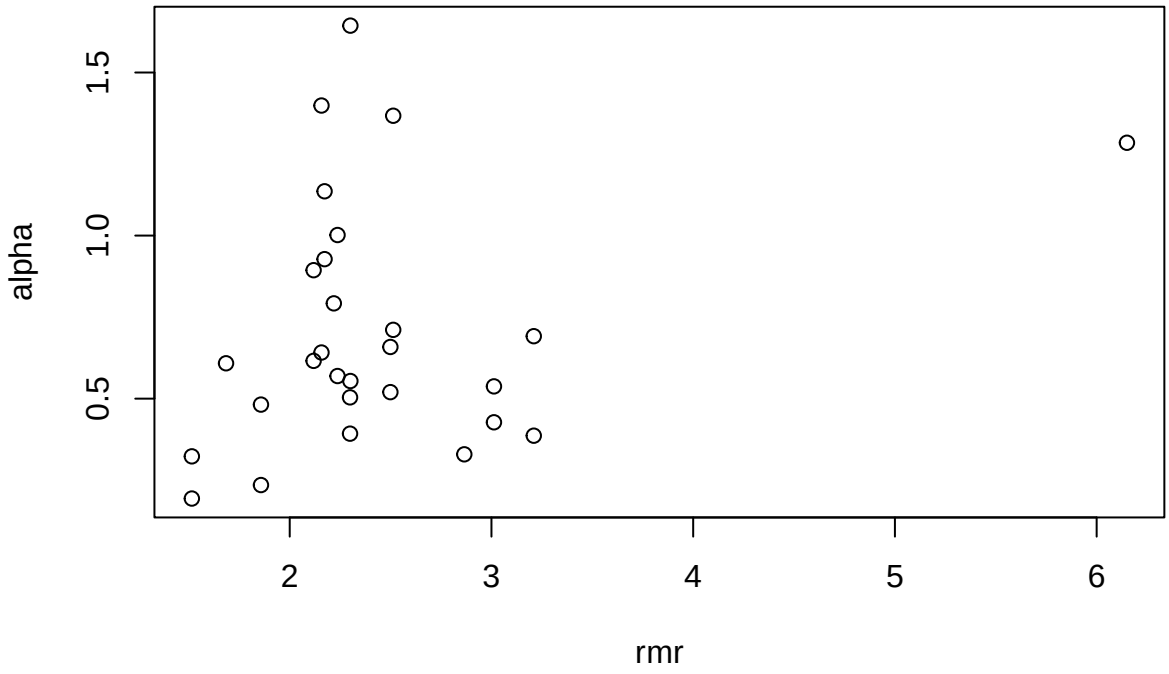
It seems the analysis could be significantly influenced by the one very high rmr of over 20. Just to cover all my bases, we should see if the linear regression is still not significant when that datum is excluded.

```
summary(aov(lm(alpha~rmr,data=pcrits[pcrits$rmr<20,])))
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## rmr          1  0.282  0.2819   2.103  0.159
## Residuals   26  3.484  0.1340
```

It is still not significantly related.

```
plot(alpha~rmr,data=pcrits[pcrits$rmr<20,])
```



11 N values for each measurement for flowchart

```
sum(pcrits$pco2==1000&pcrits$day==1)
```

```
## [1] 7
```

```
sum(pcrits$pco2==1000&pcrits$day==7)
```

```
## [1] 7
```

```
sum(pcrits$pco2==1800&pcrits$day==1)
```

```
## [1] 8
```

```
sum(pcrits$pco2==1800&pcrits$day==7)
```

```
## [1] 7
```