Critical Oxygen Pressure (P_{CRIT}) Analysis

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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")
```

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()
                  )
```

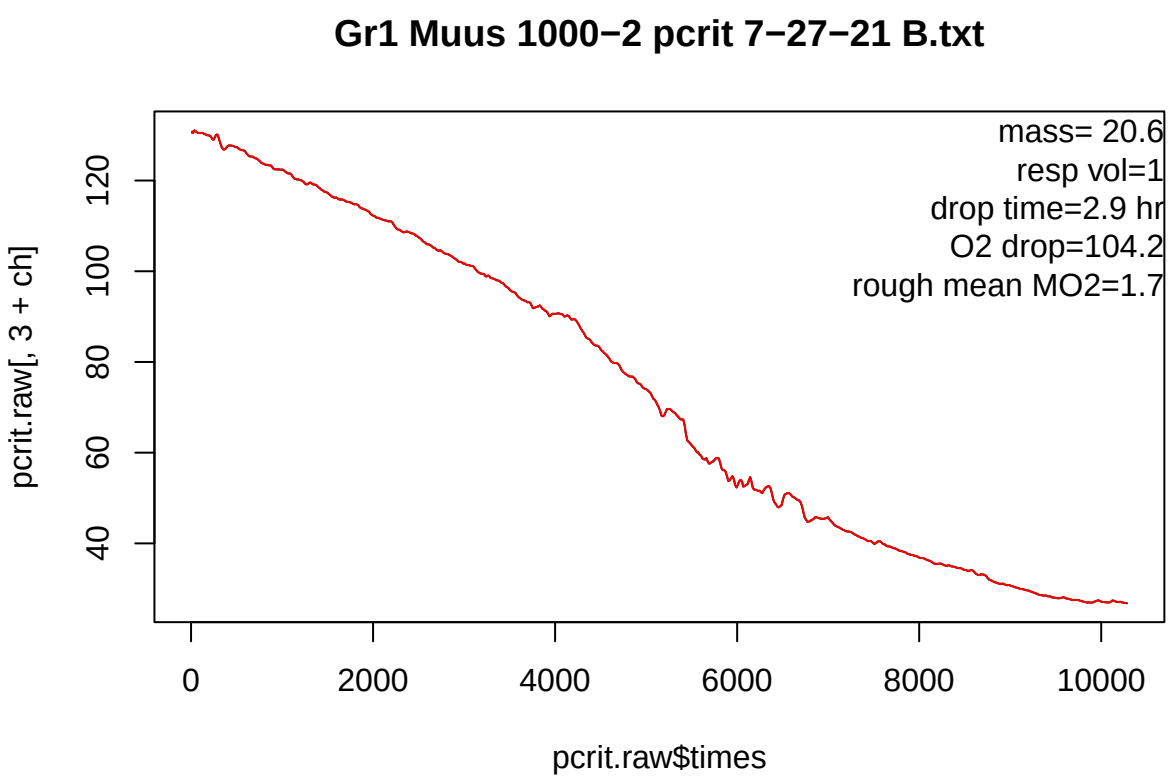
Next, I calculate the P_{CRIT} from each data file. Here are a couple of important points on our calculations:

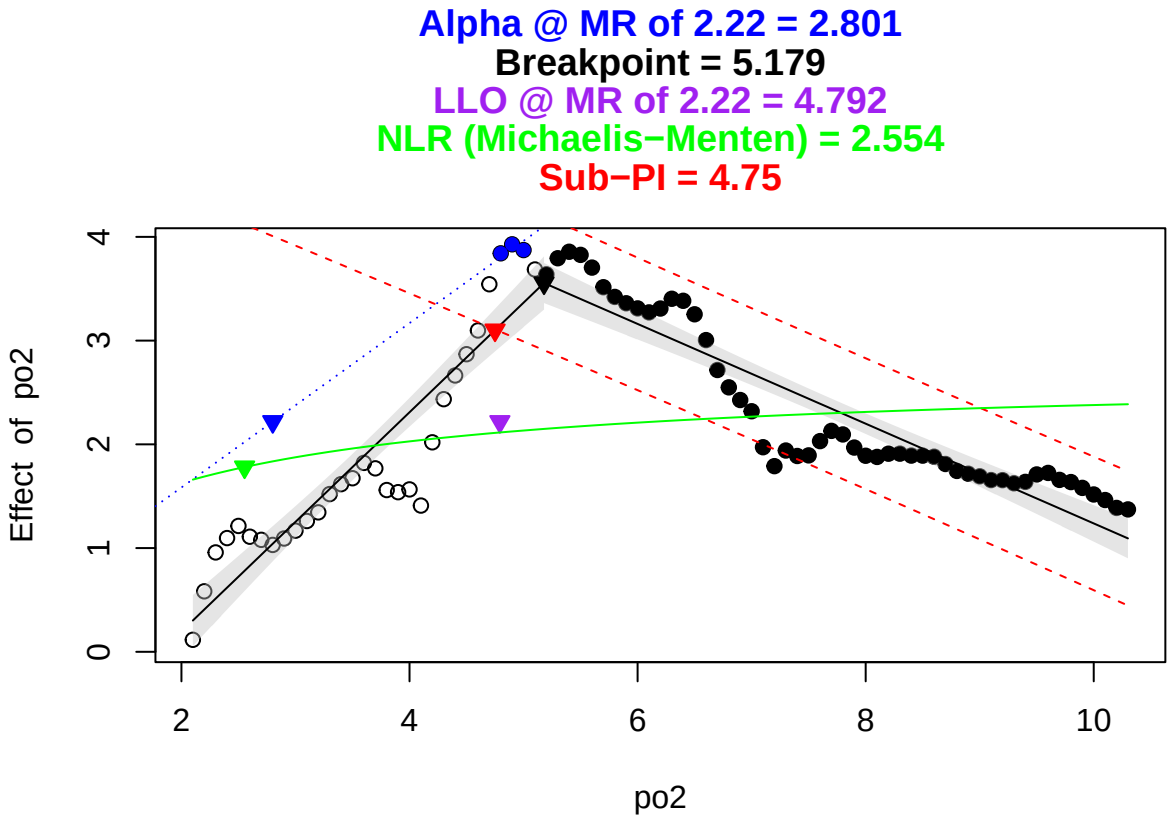
1. We are using the alpha P_{CRIT} method (Seibel et al, 2021) to calculate P_{CRIT} as implemented by the calc_pcrit() function from the "respirometry" R package (Birk, 2021).
2. We used the routine metabolic rate that we measured for each individual octopus in this study, unless the maximum metabolic 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.

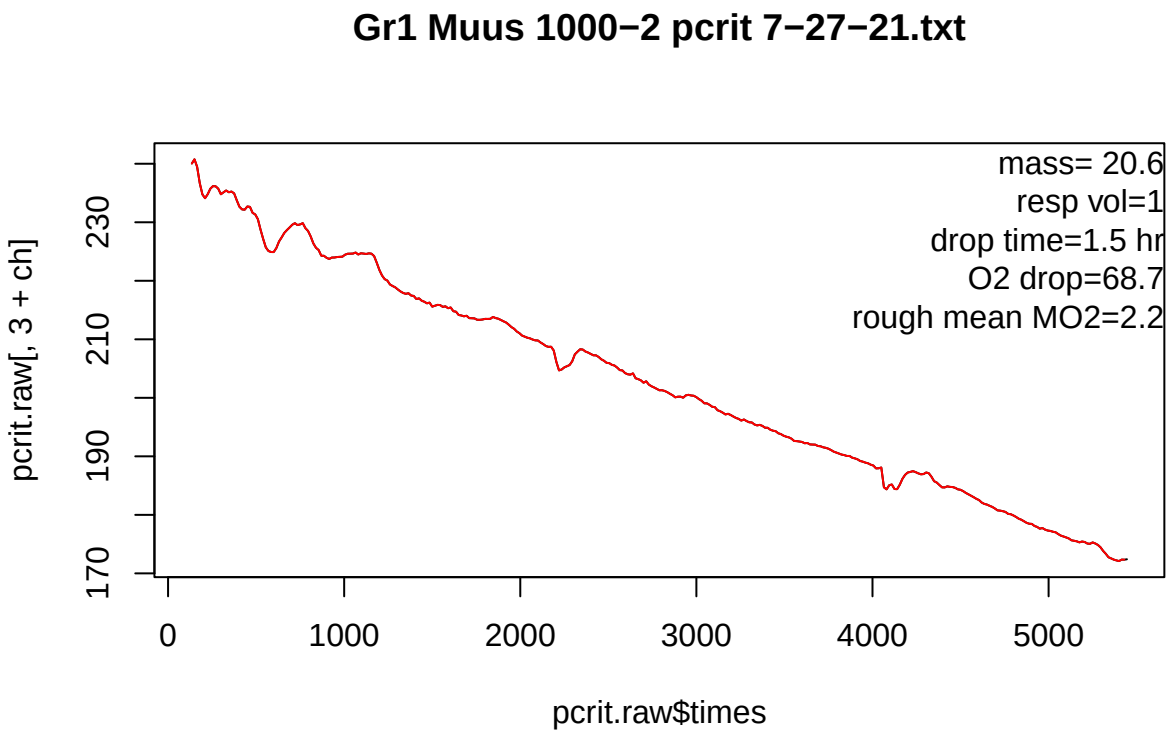
```
co=1

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&
```

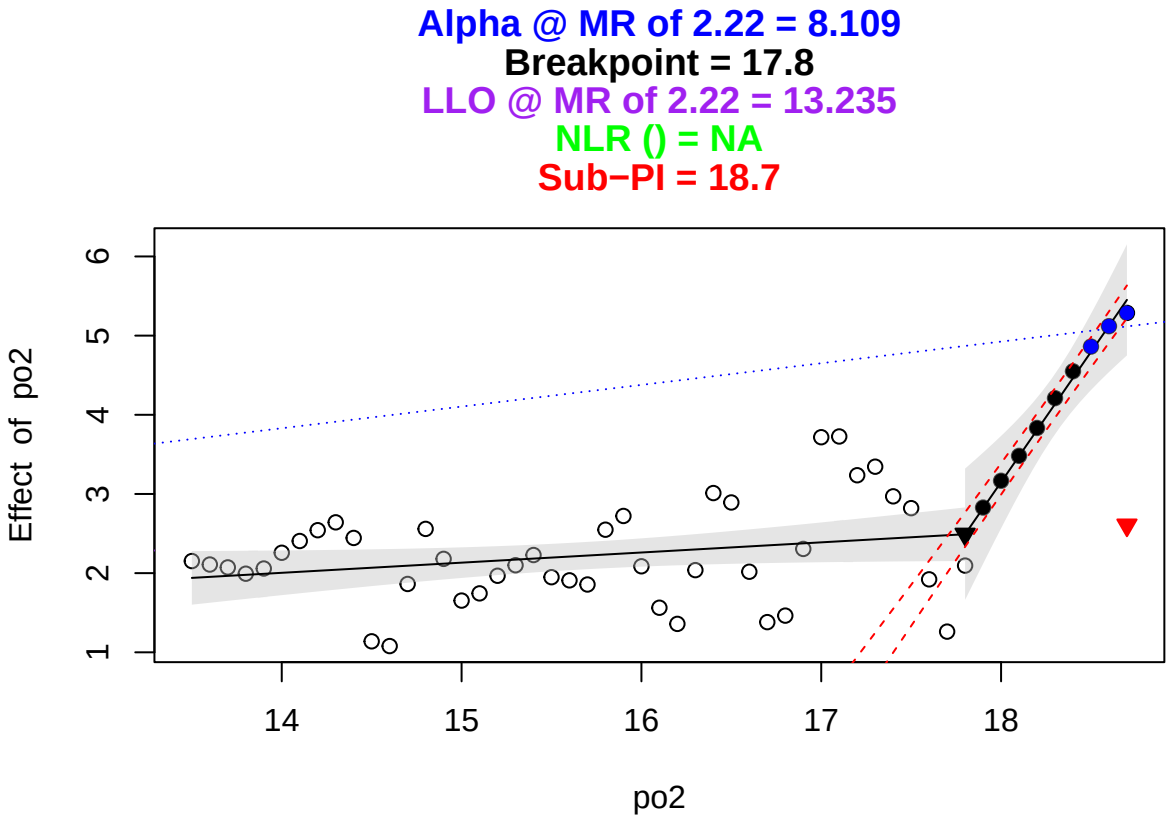
```
    pcrit.raw$times<stop,
  ]
vol=pcrit.log$vol[guess]
drop.time=round((stop-start)/3600,1)
O2.drop=round(diff(range(pcrit.working[,3+ch])),1)
rough.mo2=round((O2.drop*vol)/mass/drop.time,1)

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$resp,
           avg_top_n = 3,MR=rmr)
}else{
  plot_pcrit(pcrit.bin$po2,pcrit.bin$resp,
           avg_top_n = 3)
}


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])
  }
}else{
  pcrits[co,8]=NA
}
co=co+1

if(!is.na(pcrit.log$ch2[guess])){
  ch=pcrit.log$ch2[guess]
  octo=pcrit.log$octo2[guess]
```

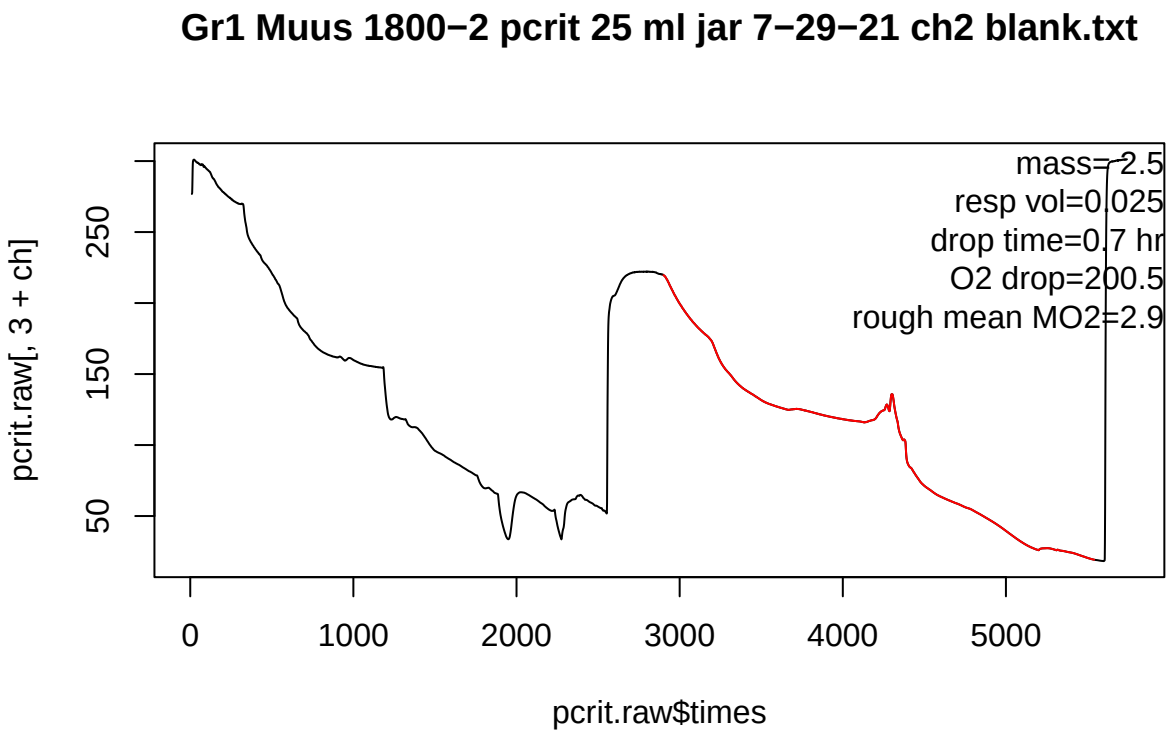
```
  start=pcrit.log[guess,6+ch]
  stop=max(pcrit.raw$times)-pcrit.log[guess,10+ch]
  mass=mean(routine$mass[routine$octo==octo])
  rmr=mean(routine$rmr[routine$octo==octo])

  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$resp,
           avg_top_n = 3,MR=rmr)
  }else{
    plot_pcrit(pcrit.bin$po2,pcrit.bin$resp,
           avg_top_n = 3)
  }

  pcrits[co,1]=filename
  pcrits[co,2]=pcrit.log$filename[guess]
  pcrits[co,3]=octo
  pcrits[co,4]=mass
  pcrits[co,5]=pcrits$pco2[guess]
  pcrits[co,6]=pcrits$day[guess]
  pcrits[co,7]=rmr
  if (min(pcrit.working[,3+ch])<50){
    if(max(pcrit.bin$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])
    }
  }else{
    pcrits[co,8]=NA
  }
  co=co+1

}


}
```

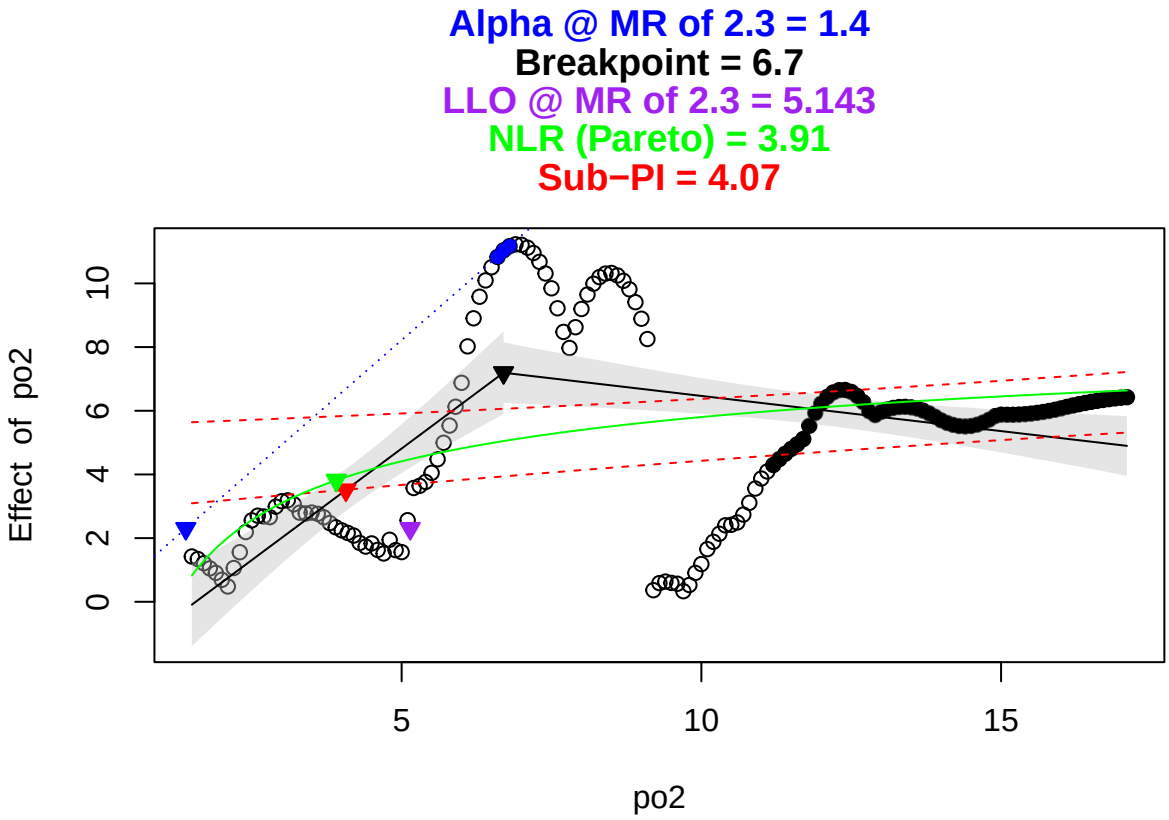

Gr1 Muus 1000−2 pcrit 7−27−21 B.txt
mass= 20.6
resp vol=1
drop time=2.9 hr
O2 drop=104.2
rough mean MO2=1.7
pcrit.raw[, 3 + ch]
40
60
80
100
120
0
2000
4000
6000
8000
10000
pcrit.raw$times

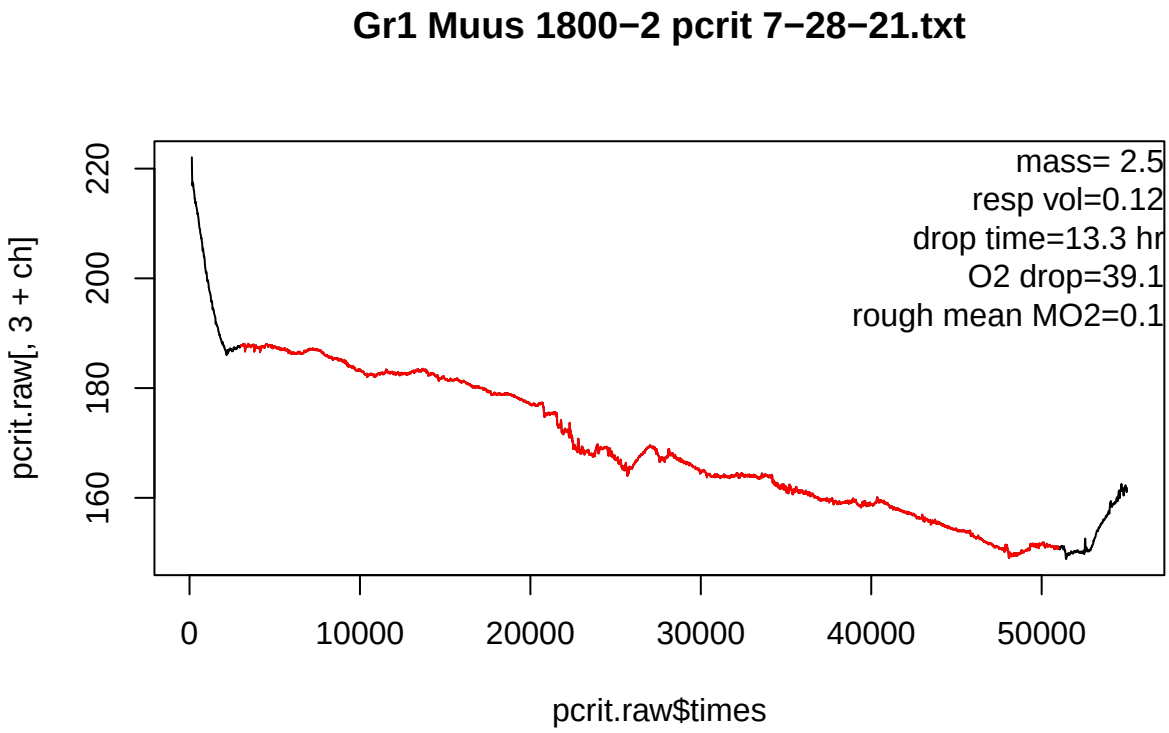

Alpha @ MR of 2.22 = 2.801
Breakpoint = 5.179
LLO @ MR of 2.22 = 4.792
NLR (Michaelis−Menten) = 2.554
Sub−PI = 4.75
Effect of po2
po2
0
1
2
3
4
2
4
6
8
10

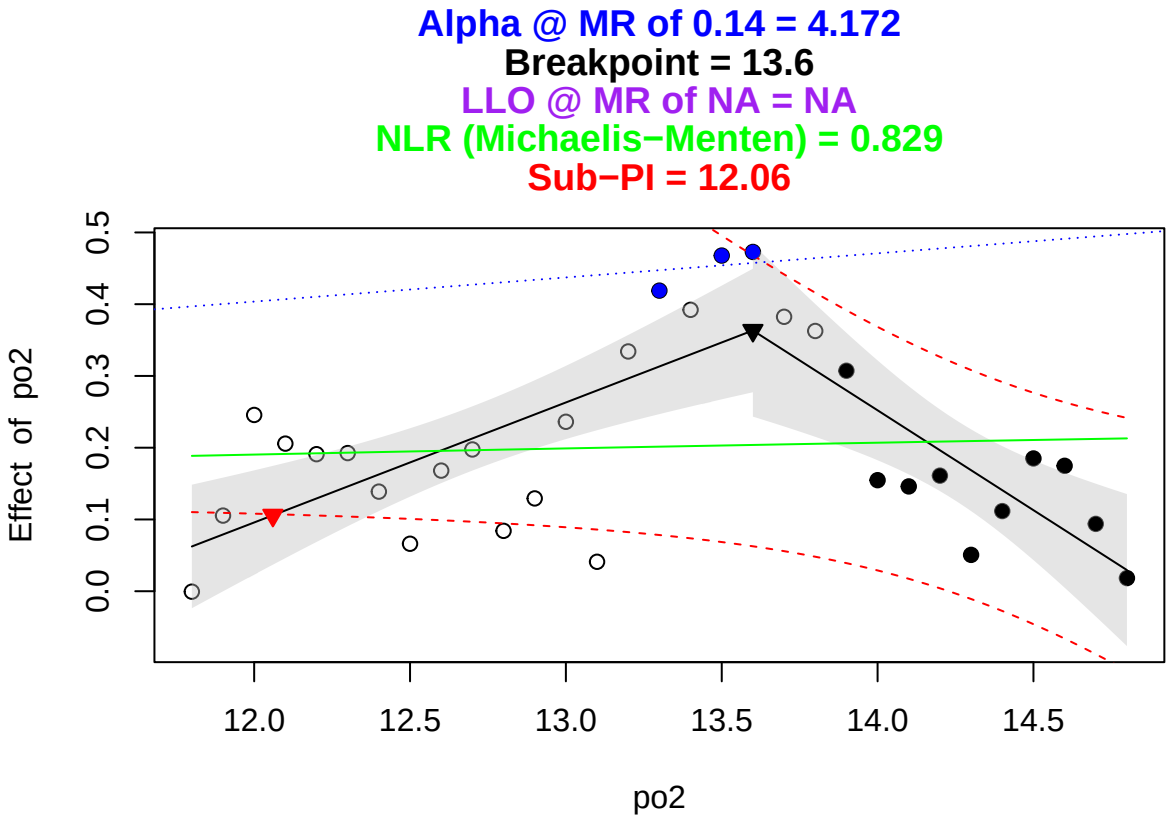

Gr1 Muus 1000−2 pcrit 7−27−21.txt
mass= 20.6
resp vol=1
drop time=1.5 hr
O2 drop=68.7
rough mean MO2=2.2
pcrit.raw[, 3 + ch]
170
190
210
230
0
1000
2000
3000
4000
5000
pcrit.raw$times

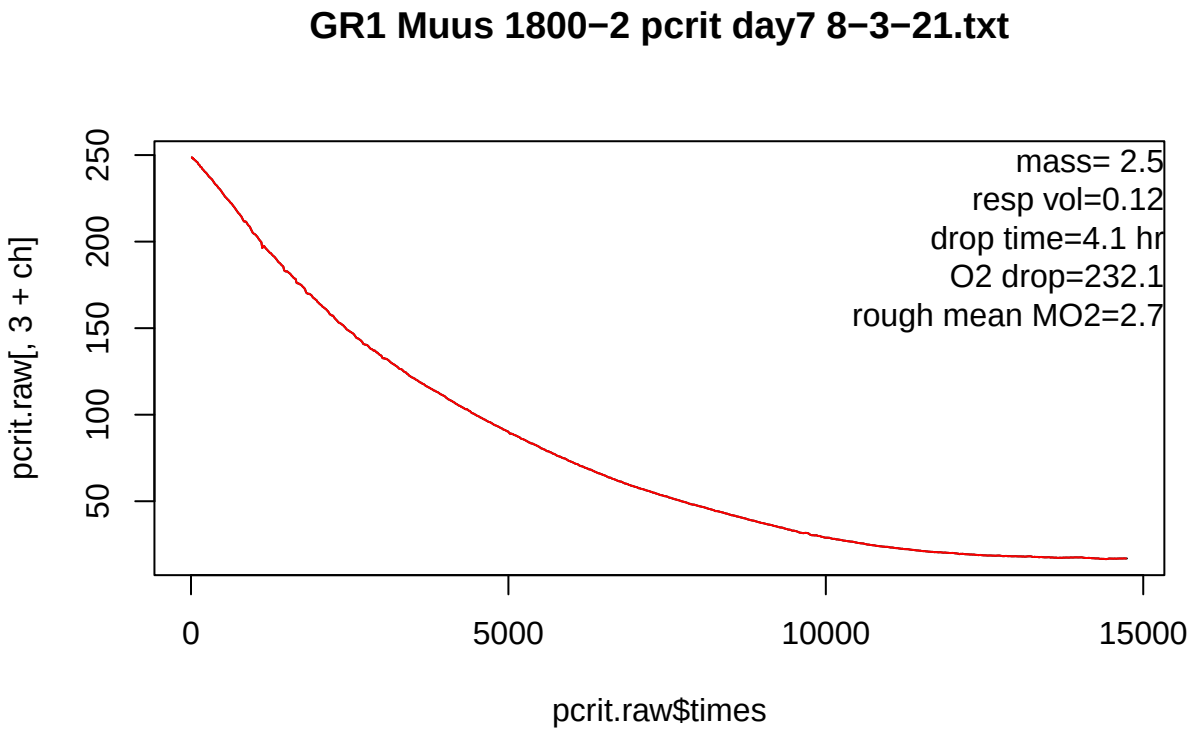

Alpha @ MR of 2.22 = 8.109
Breakpoint = 17.8
LLO @ MR of 2.22 = 13.235
NLR () = NA
Sub−PI = 18.7
Effect of po2
1
2
3
4
5
6
14
15
16
17
18
po2

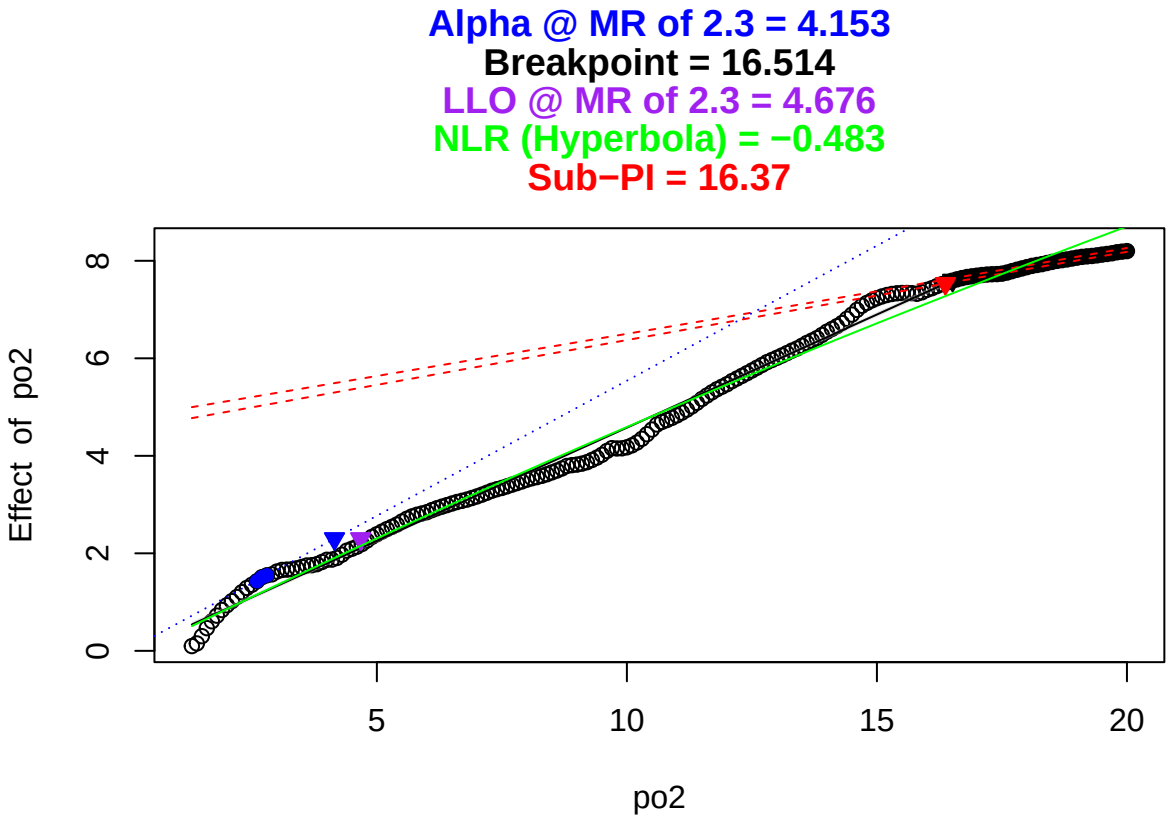

Gr1 Muus 1800−2 pcrit 25 ml jar 7−29−21 ch2 blank.txt
mass= 2.5
resp vol=0.025
drop time=0.7 hr
O2 drop=200.5
rough mean MO2=2.9
pcrit.raw[, 3 + ch]
50
150
250
0
1000
2000
3000
4000
5000
pcrit.raw$times

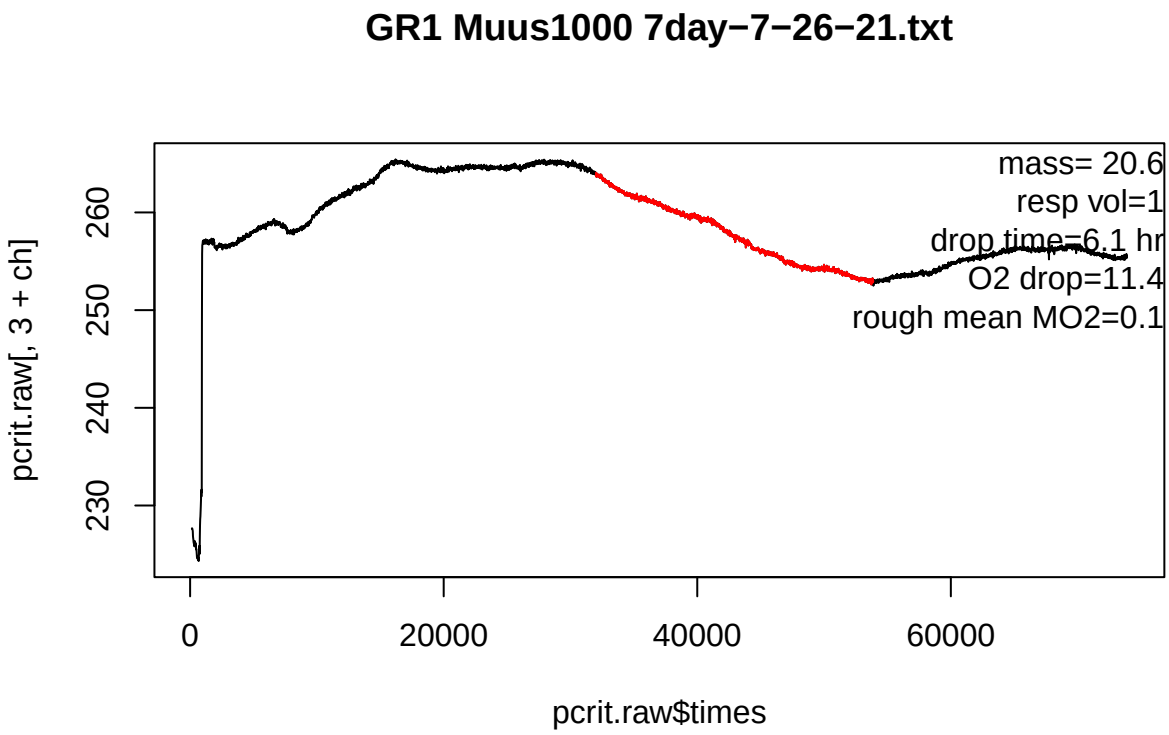

Alpha @ MR of 2.3 = 1.4
Breakpoint = 6.7
LLO @ MR of 2.3 = 5.143
NLR (Pareto) = 3.91
Sub−PI = 4.07
Effect of po2
0
2
4
6
8
10
5
10
15
po2

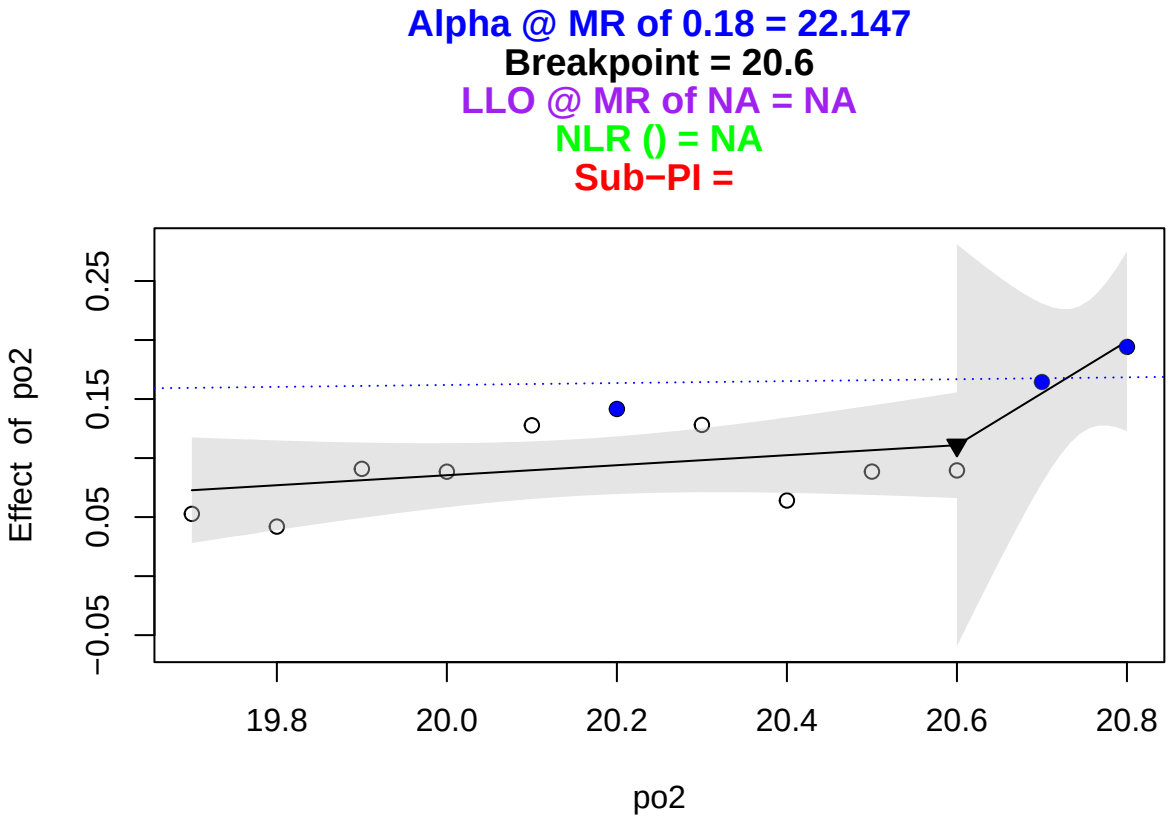

Gr1 Muus 1800−2 pcrit 7−28−21.txt
mass= 2.5
resp vol=0.12
drop time=13.3 hr
O2 drop=39.1
rough mean MO2=0.1
pcrit.raw[, 3 + ch]
160
180
200
220
0
10000
20000
30000
40000
50000
pcrit.raw$times

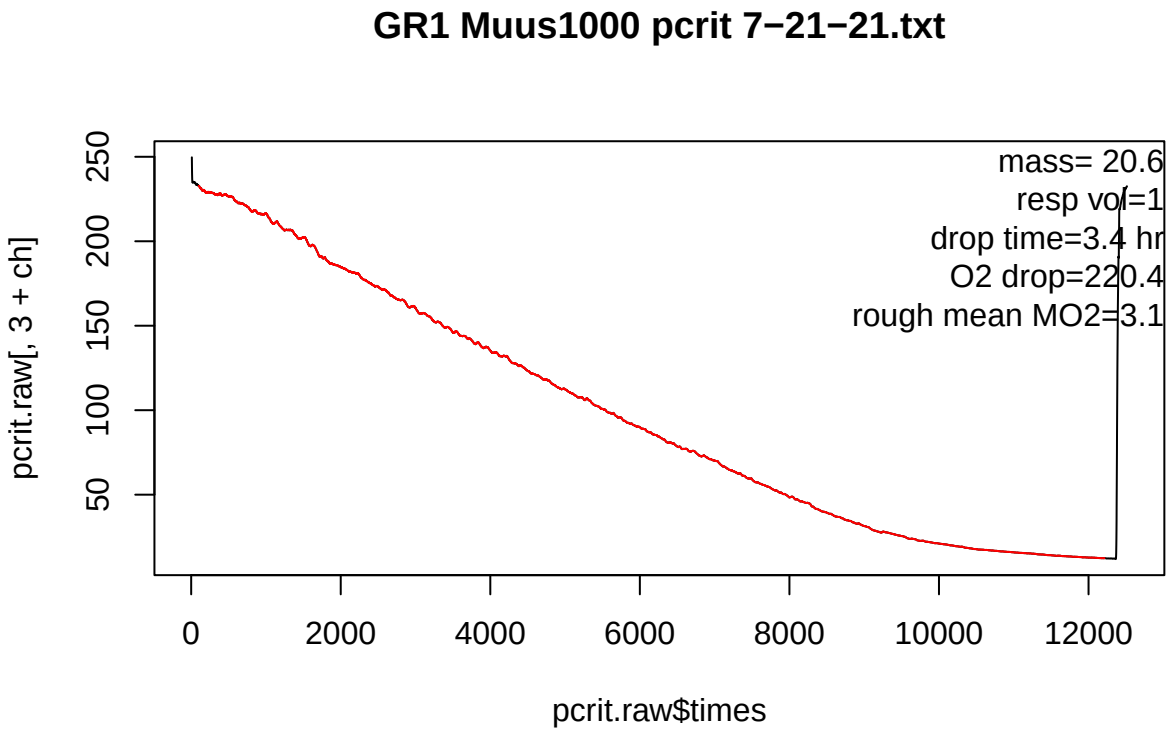

Alpha @ MR of 0.14 = 4.172
Breakpoint = 13.6
LLO @ MR of NA = NA
NLR (Michaelis−Menten) = 0.829
Sub−PI = 12.06
Effect of po2
0.0
0.1
0.2
0.3
0.4
0.5
12.0
12.5
13.0
13.5
14.0
14.5
po2

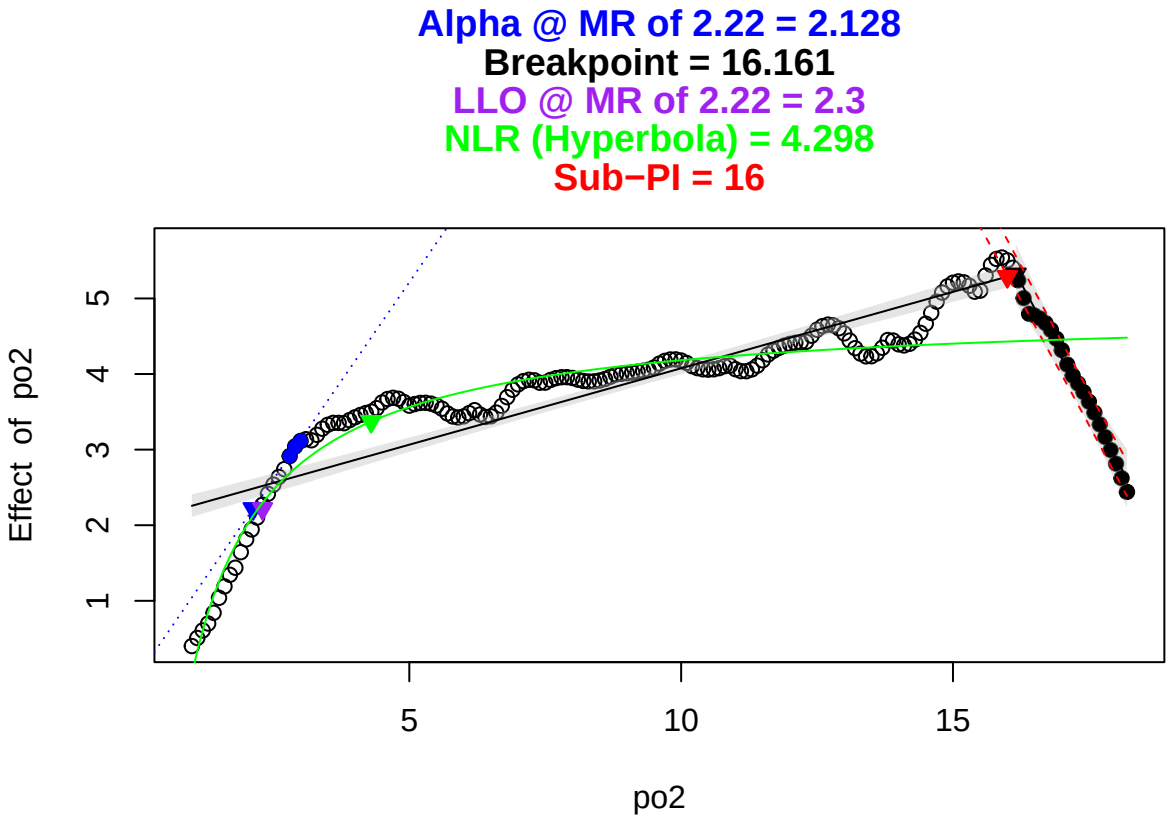

GR1 Muus 1800−2 pcrit day7 8−3−21.txt
mass= 2.5
resp vol=0.12
drop time=4.1 hr
O2 drop=232.1
rough mean MO2=2.7
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
pcrit.raw$times

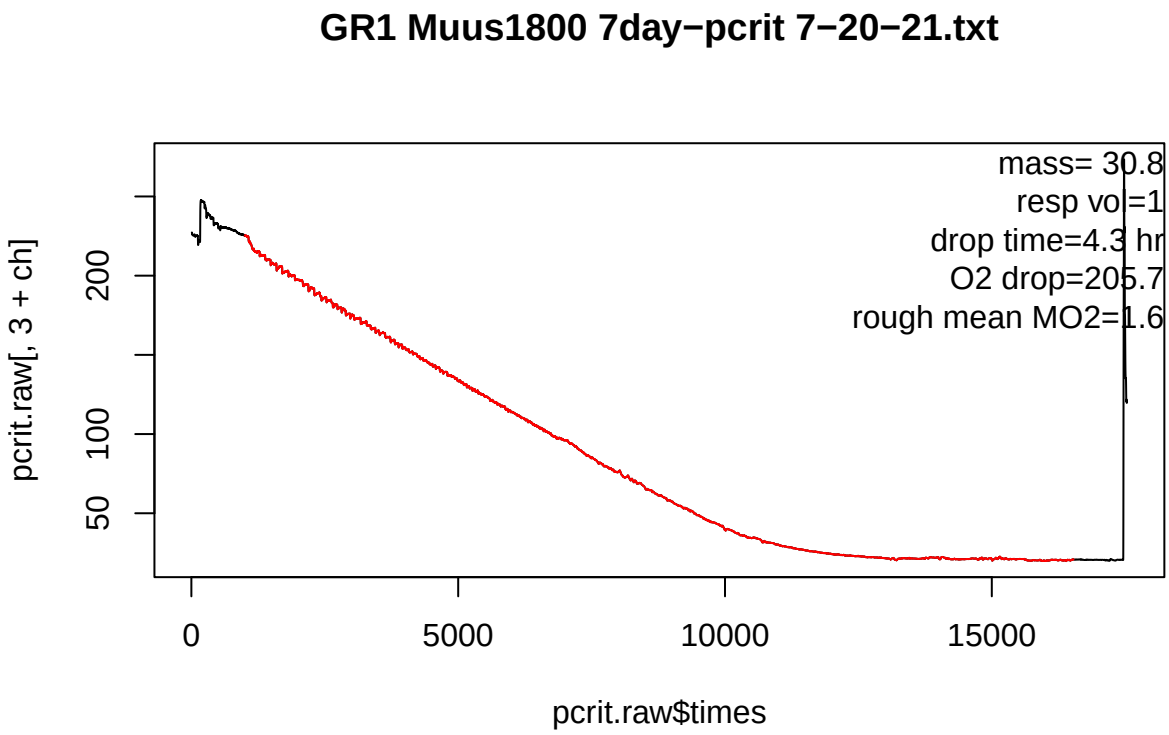

Alpha @ MR of 2.3 = 4.153
Breakpoint = 16.514
LLO @ MR of 2.3 = 4.676
NLR (Hyperbola) = −0.483
Sub−PI = 16.37
Effect of po2
0
2
4
6
8
5
10
15
20
po2

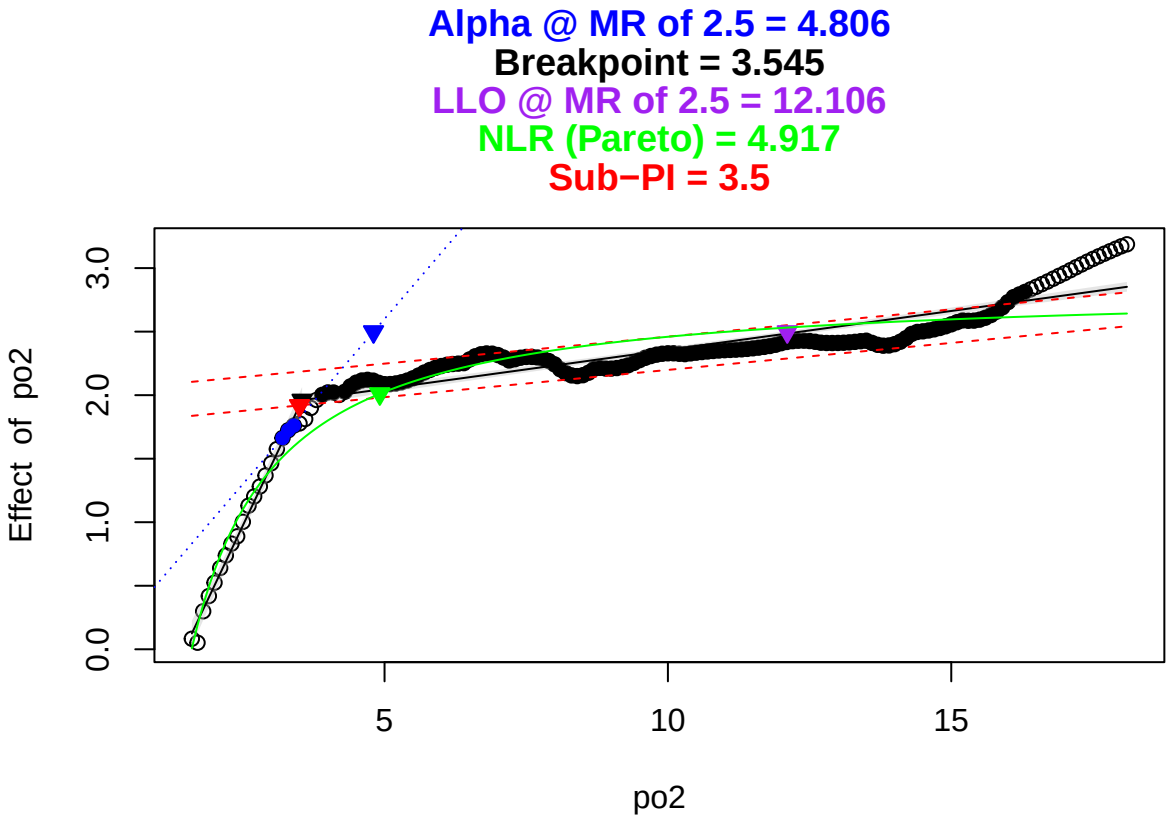

GR1 Muus1000 7day−7−26−21.txt
mass= 20.6
resp vol=1
drop time=6.1 hr
O2 drop=11.4
rough mean MO2=0.1
pcrit.raw[, 3 + ch]
230
240
250
260
0
20000
40000
60000
pcrit.raw$times

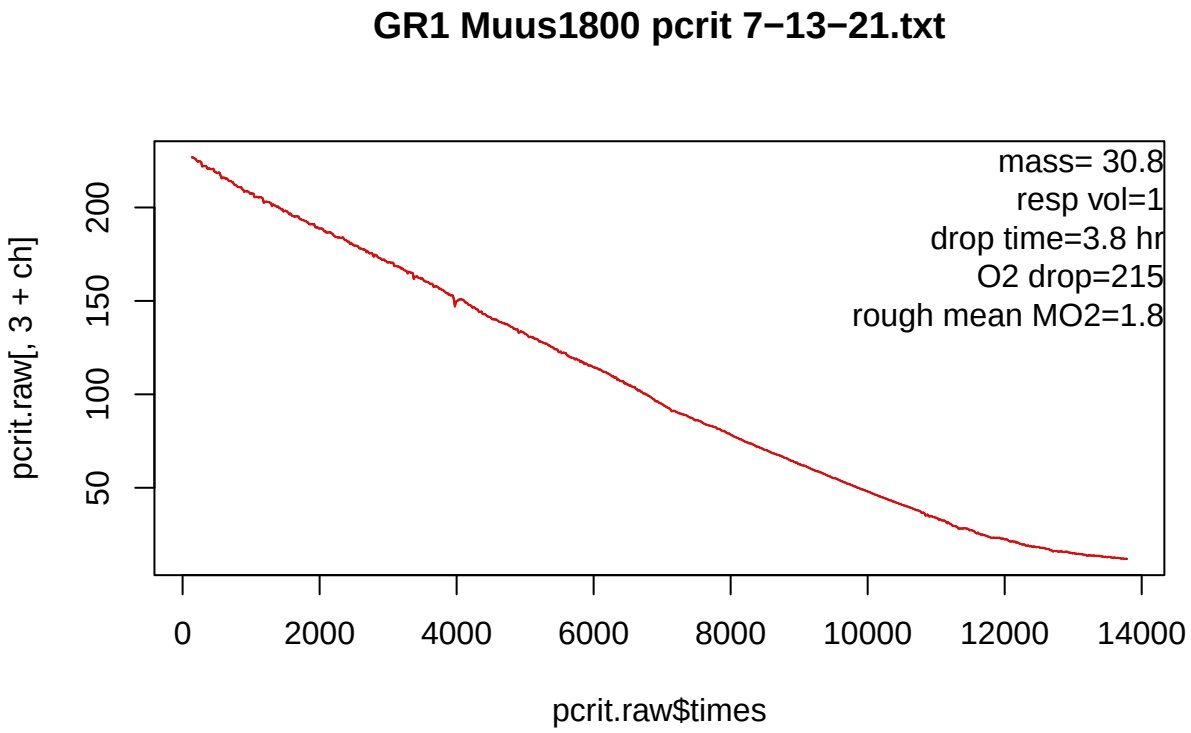

Alpha @ MR of 0.18 = 22.147
Breakpoint = 20.6
LLO @ MR of NA = NA
NLR () = NA
Sub−PI =
Effect of po2
0.25
0.15
0.05
−0.05
19.8
20.0
20.2
20.4
20.6
20.8
po2

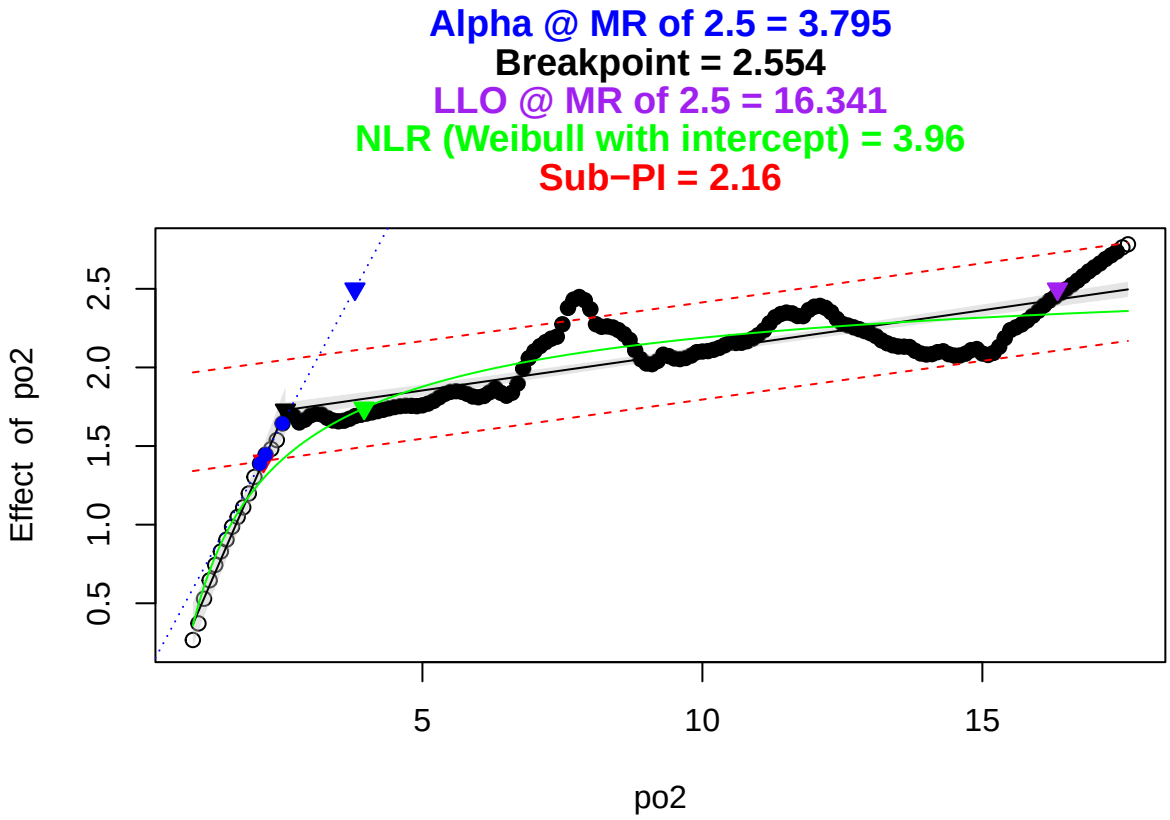

GR1 Muus1000 pcrit 7−21−21.txt
mass= 20.6
resp vol=1
drop time=3.4 hr
O2 drop=220.4
rough mean MO2=3.1
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
2000
4000
6000
8000
10000
12000
pcrit.raw$times

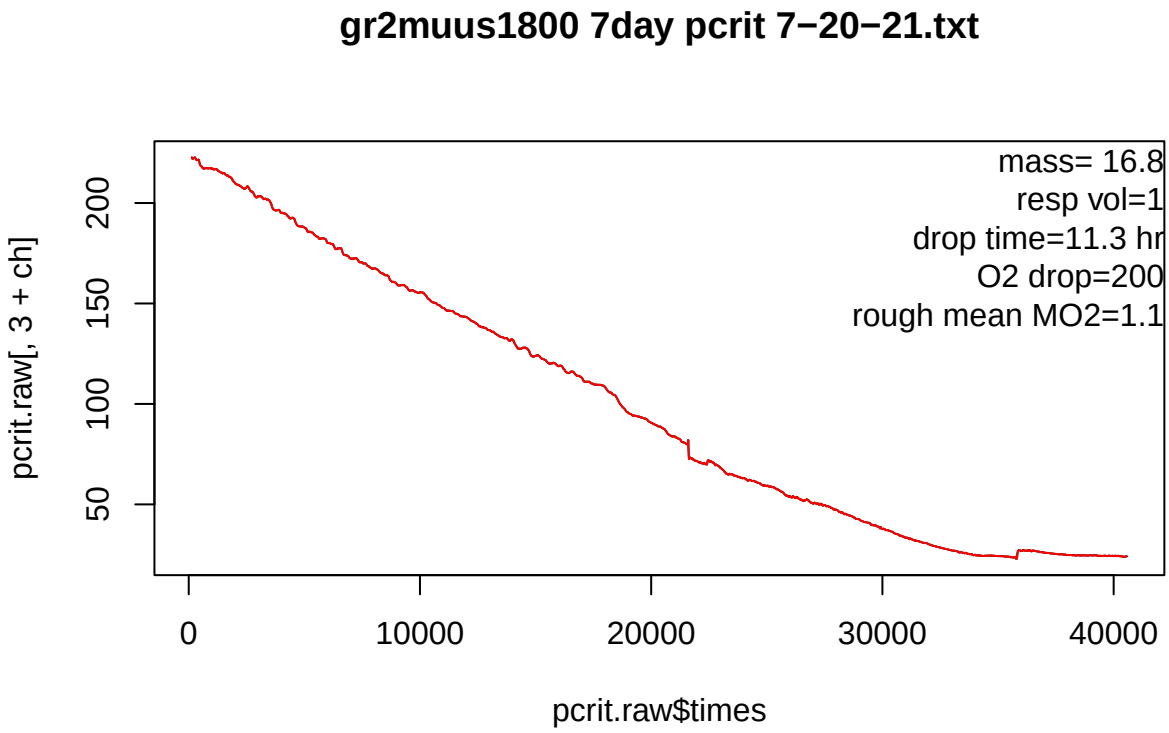

Alpha @ MR of 2.22 = 2.128
Breakpoint = 16.161
LLO @ MR of 2.22 = 2.3
NLR (Hyperbola) = 4.298
Sub−PI = 16
Effect of po2
1
2
3
4
5
5
10
15
po2

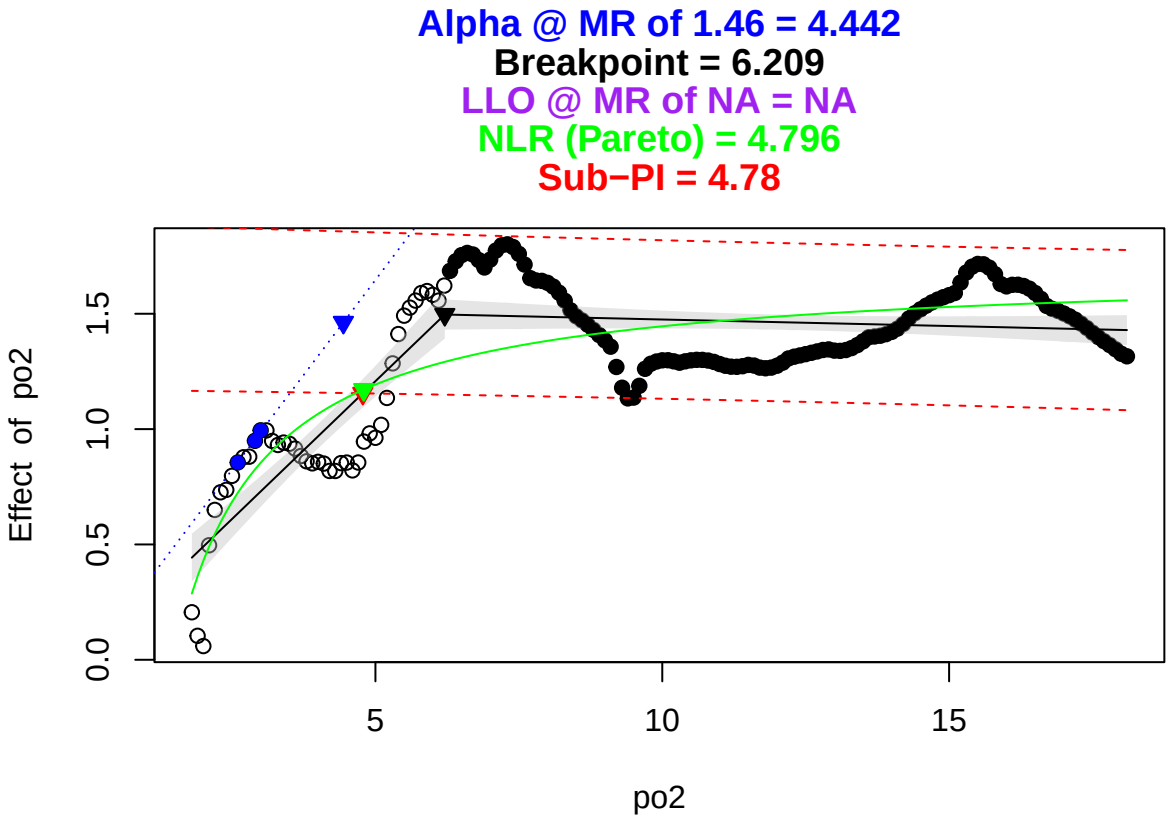

GR1 Muus1800 7day−pcrit 7−20−21.txt
mass= 30.8
resp vol=1
drop time=4.3 hr
O2 drop=205.7
rough mean MO2=1.6
pcrit.raw[, 3 + ch]
50
100
200
0
5000
10000
15000
pcrit.raw$times

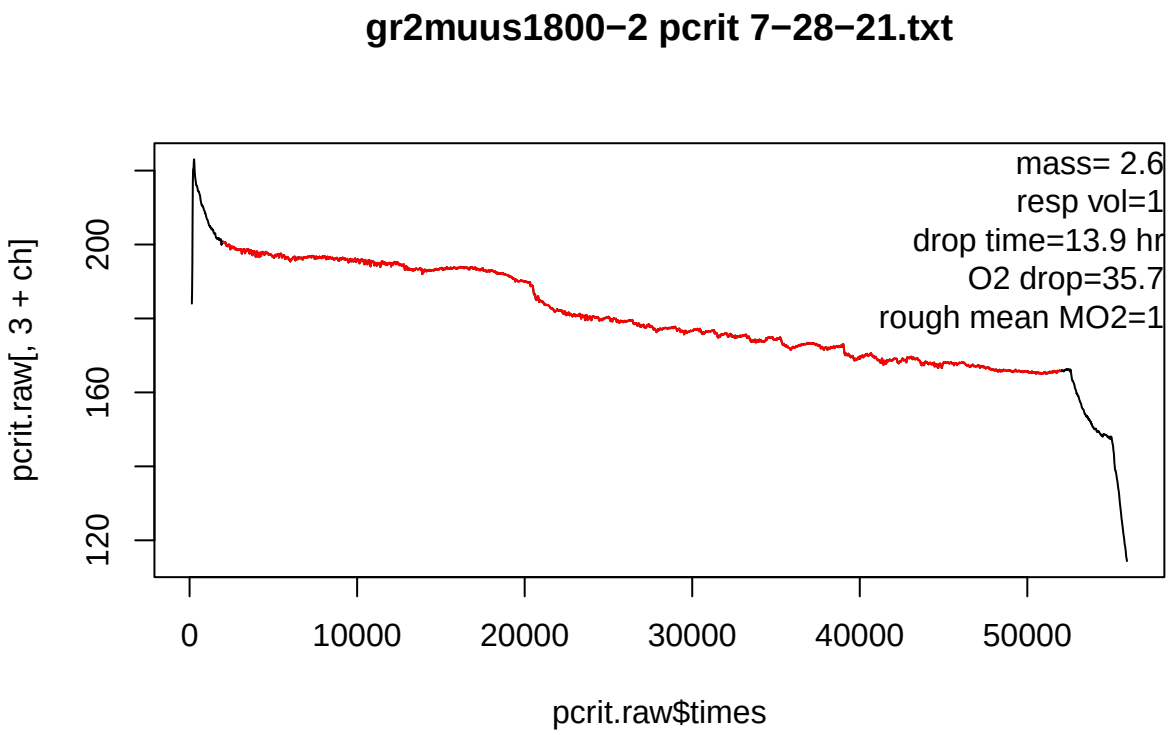

Alpha @ MR of 2.5 = 4.806
Breakpoint = 3.545
LLO @ MR of 2.5 = 12.106
NLR (Pareto) = 4.917
Sub−PI = 3.5
Effect of po2
0.0
1.0
2.0
3.0
5
10
15
po2

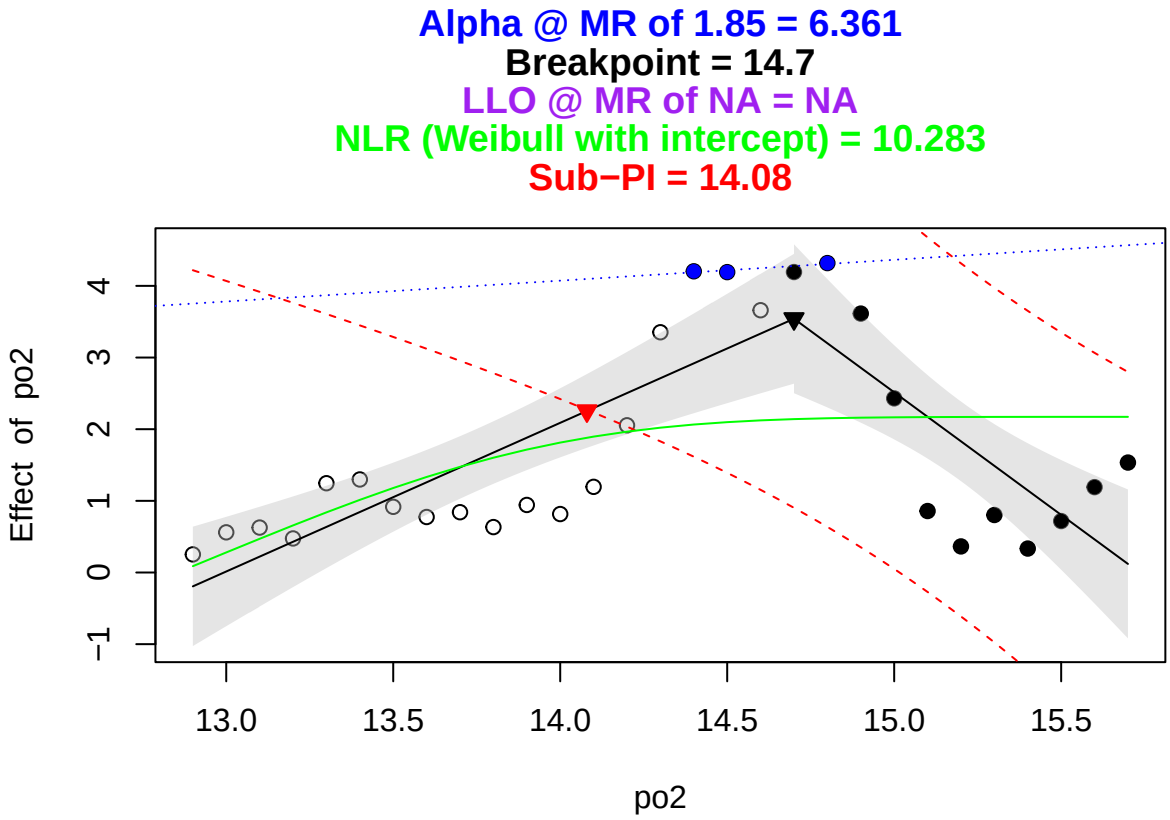

GR1 Muus1800 pcrit 7−13−21.txt
mass= 30.8
resp vol=1
drop time=3.8 hr
O2 drop=215
rough mean MO2=1.8
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
10000
12000
14000
pcrit.raw$times

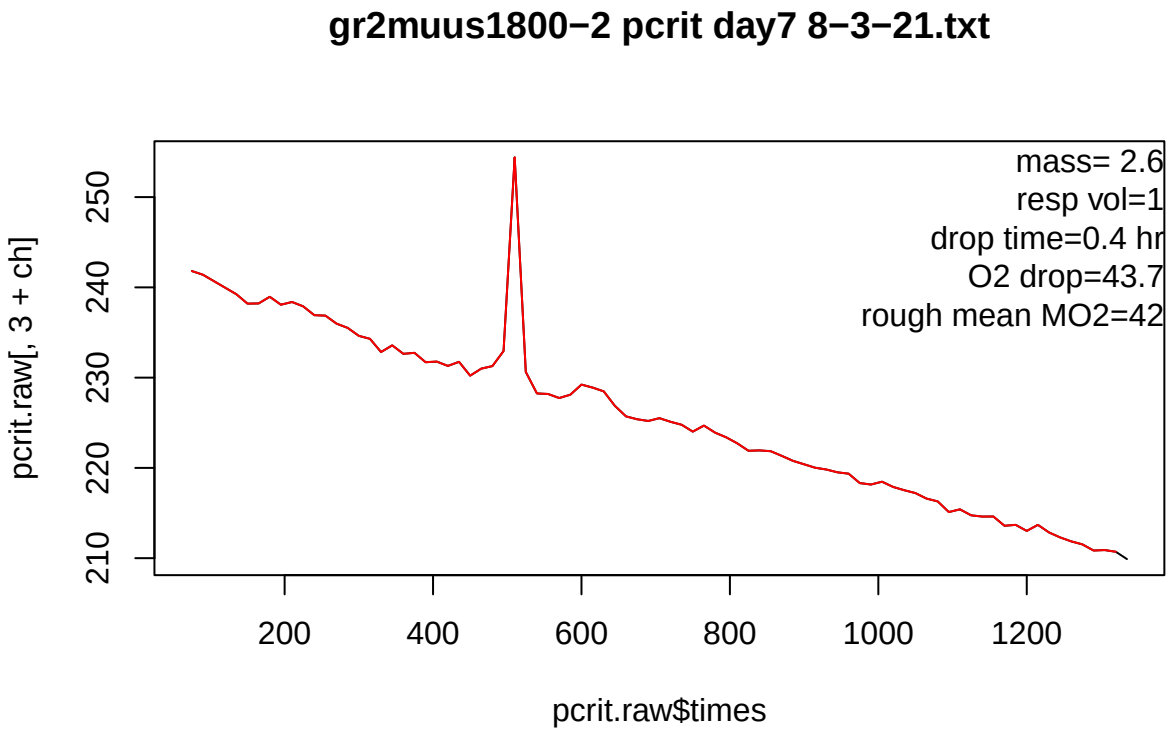

Alpha @ MR of 2.5 = 3.795
Breakpoint = 2.554
LLO @ MR of 2.5 = 16.341
NLR (Weibull with intercept) = 3.96
Sub−PI = 2.16
Effect of po2
0.5
1.0
1.5
2.0
2.5
5
10
15
po2

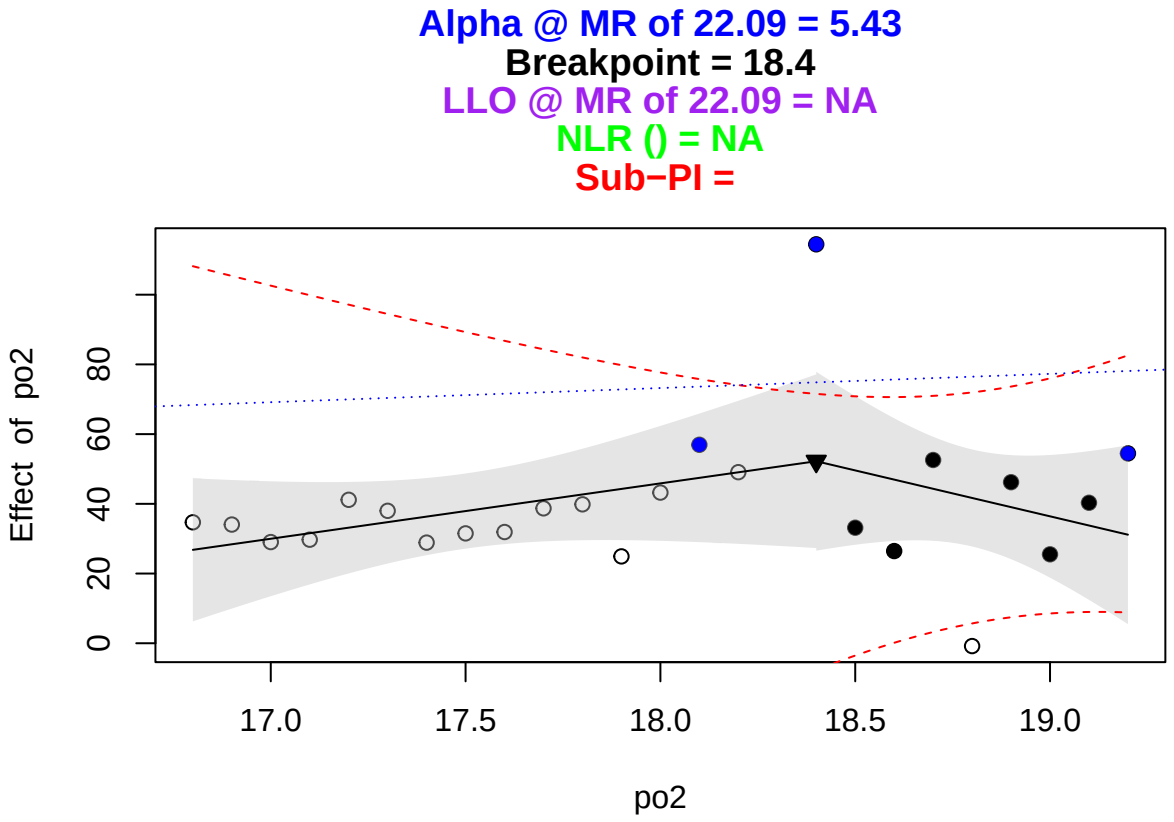

gr2muus1800 7day pcrit 7−20−21.txt
mass= 16.8
resp vol=1
drop time=11.3 hr
O2 drop=200
rough mean MO2=1.1
pcrit.raw[, 3 + ch]
50
100
150
200
0
10000
20000
30000
40000
pcrit.raw$times

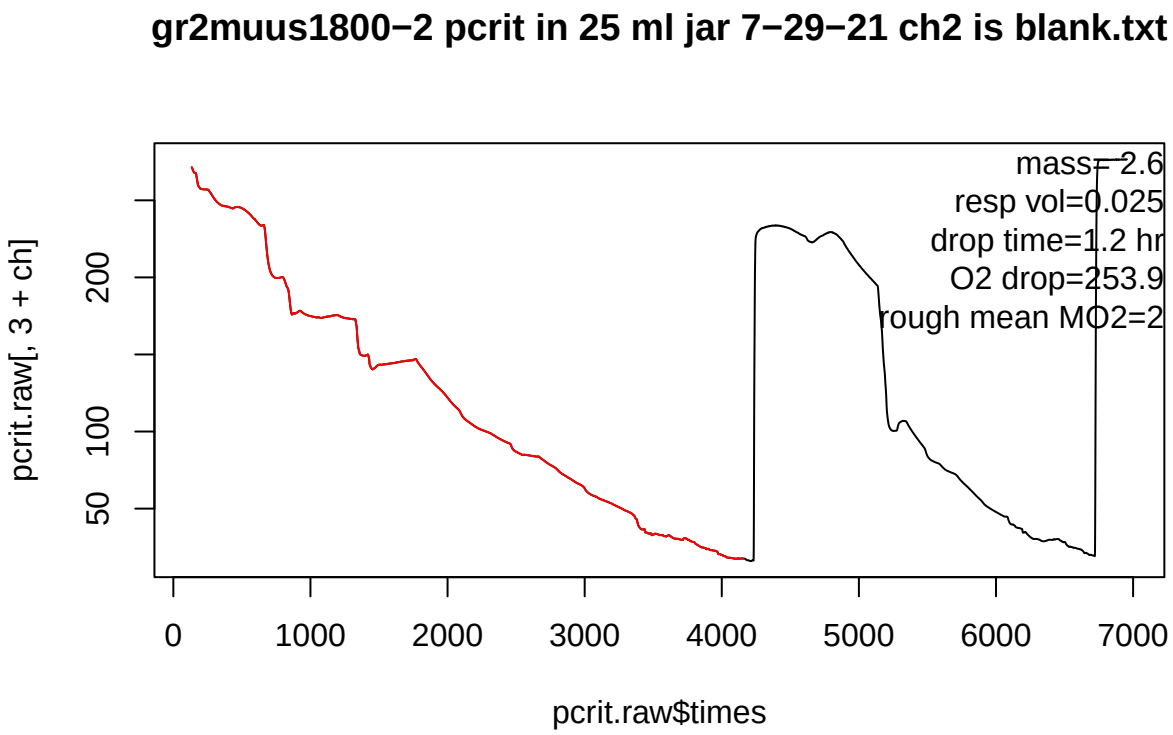

Alpha @ MR of 1.46 = 4.442
Breakpoint = 6.209
LLO @ MR of NA = NA
NLR (Pareto) = 4.796
Sub−PI = 4.78
Effect of po2
0.0
0.5
1.0
1.5
5
10
15
po2

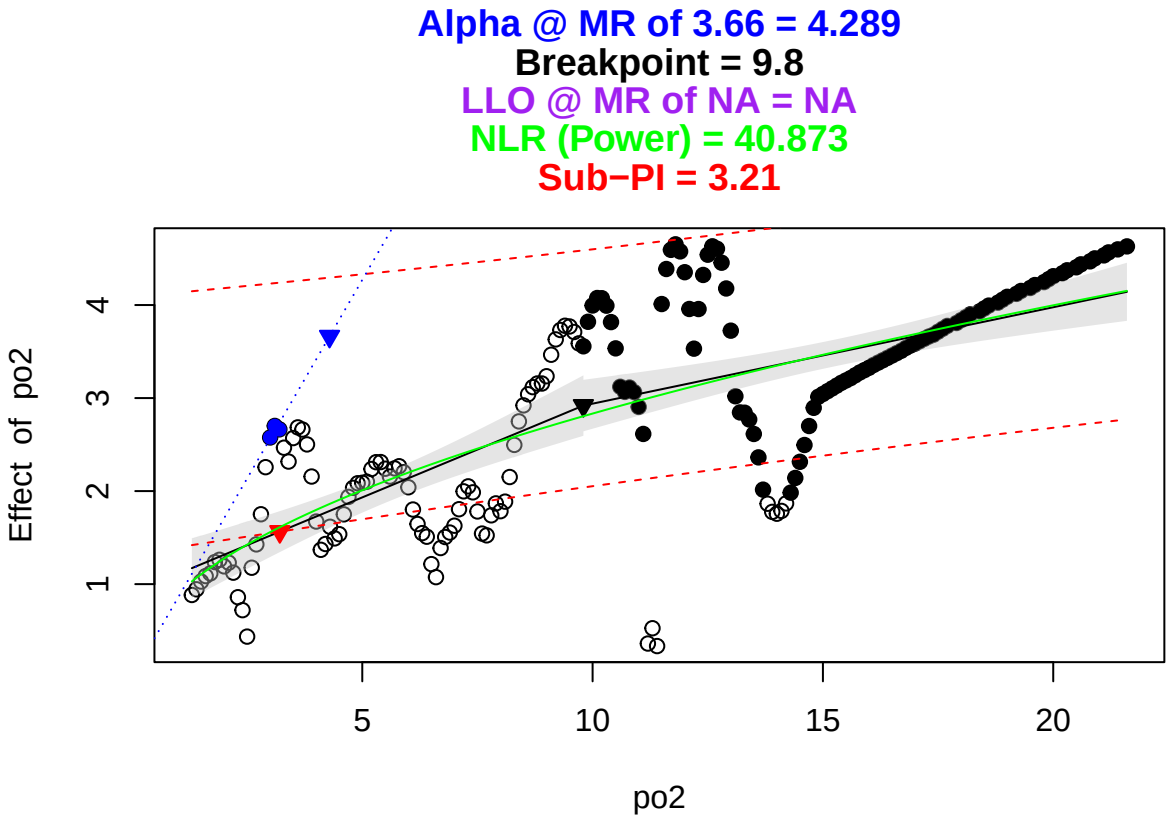

gr2muus1800−2 pcrit 7−28−21.txt
mass= 2.6
resp vol=1
drop time=13.9 hr
O2 drop=35.7
rough mean MO2=1
pcrit.raw[, 3 + ch]
120
160
200
0
10000
20000
30000
40000
50000
pcrit.raw$times

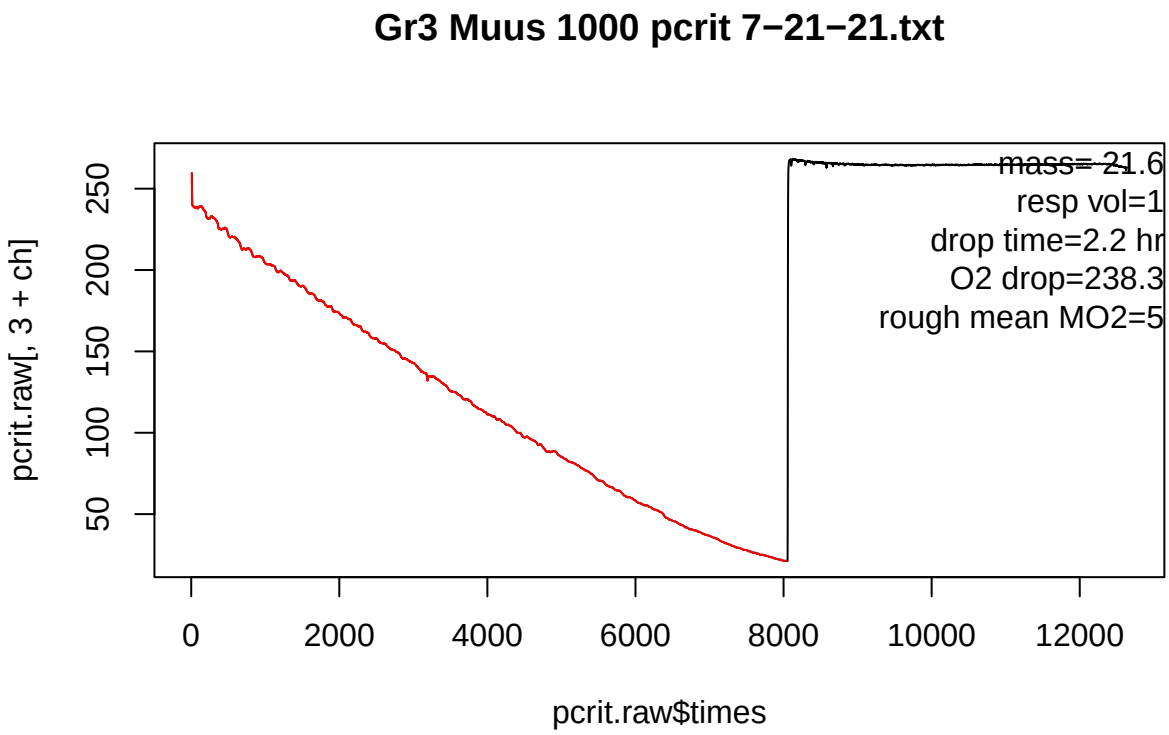

Alpha @ MR of 1.85 = 6.361
Breakpoint = 14.7
LLO @ MR of NA = NA
NLR (Weibull with intercept) = 10.283
Sub−PI = 14.08
Effect of po2
−1
0
1
2
3
4
13.0
13.5
14.0
14.5
15.0
15.5
po2

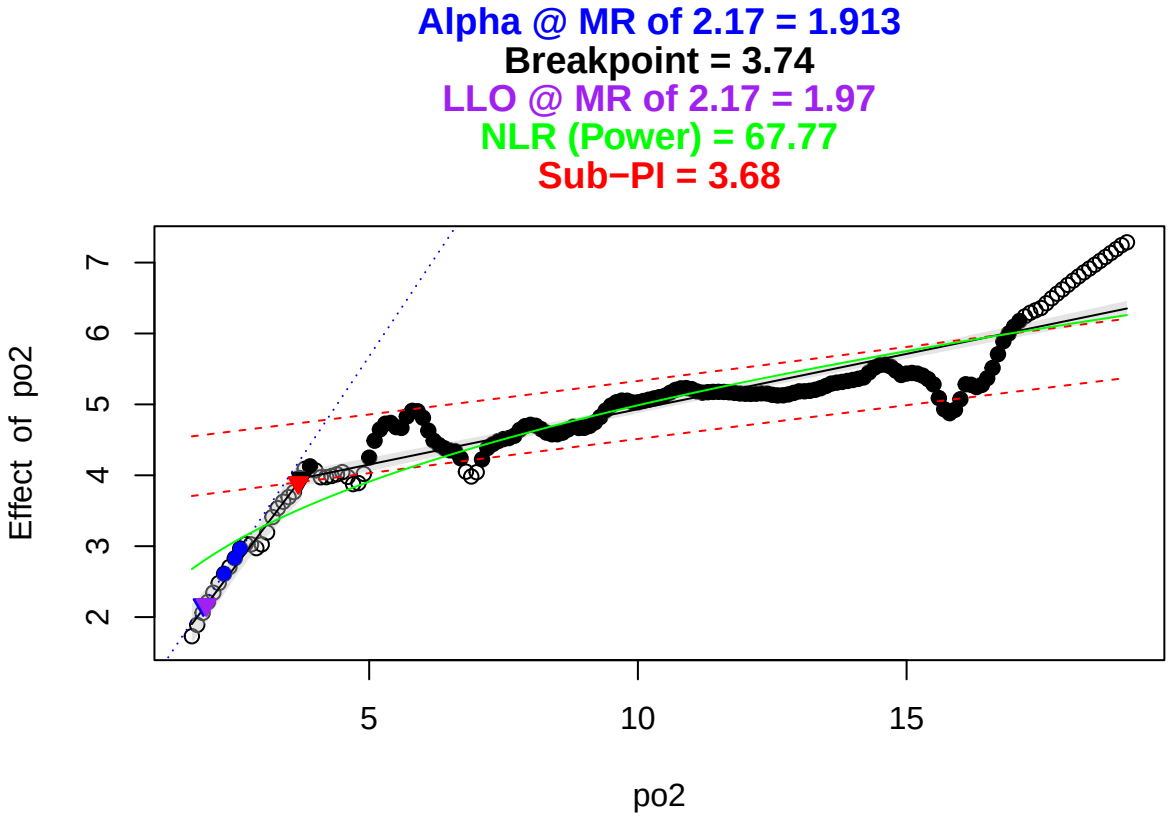

gr2muus1800−2 pcrit day7 8−3−21.txt
mass= 2.6
resp vol=1
drop time=0.4 hr
O2 drop=43.7
rough mean MO2=42
pcrit.raw[, 3 + ch]
210
220
230
240
250
200
400
600
800
1000
1200
pcrit.raw$times

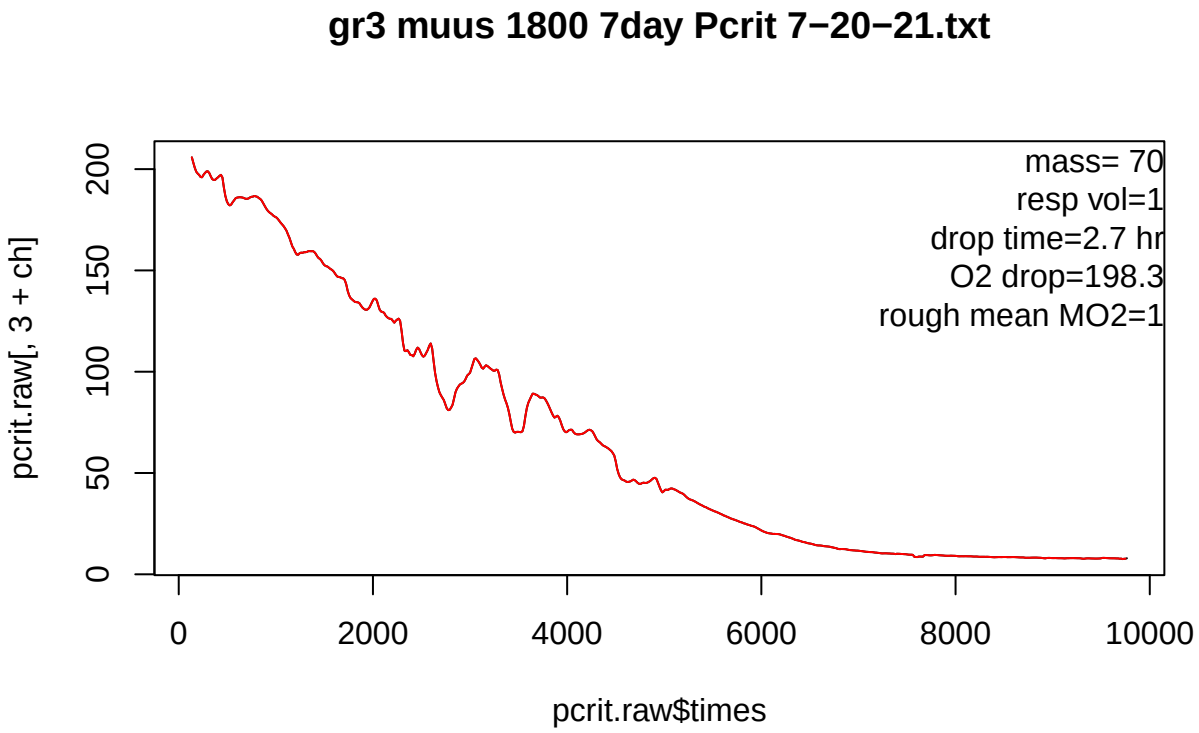

Alpha @ MR of 22.09 = 5.43
Breakpoint = 18.4
LLO @ MR of 22.09 = NA
NLR () = NA
Sub−PI =
Effect of po2
0
20
40
60
80
17.0
17.5
18.0
18.5
19.0
po2

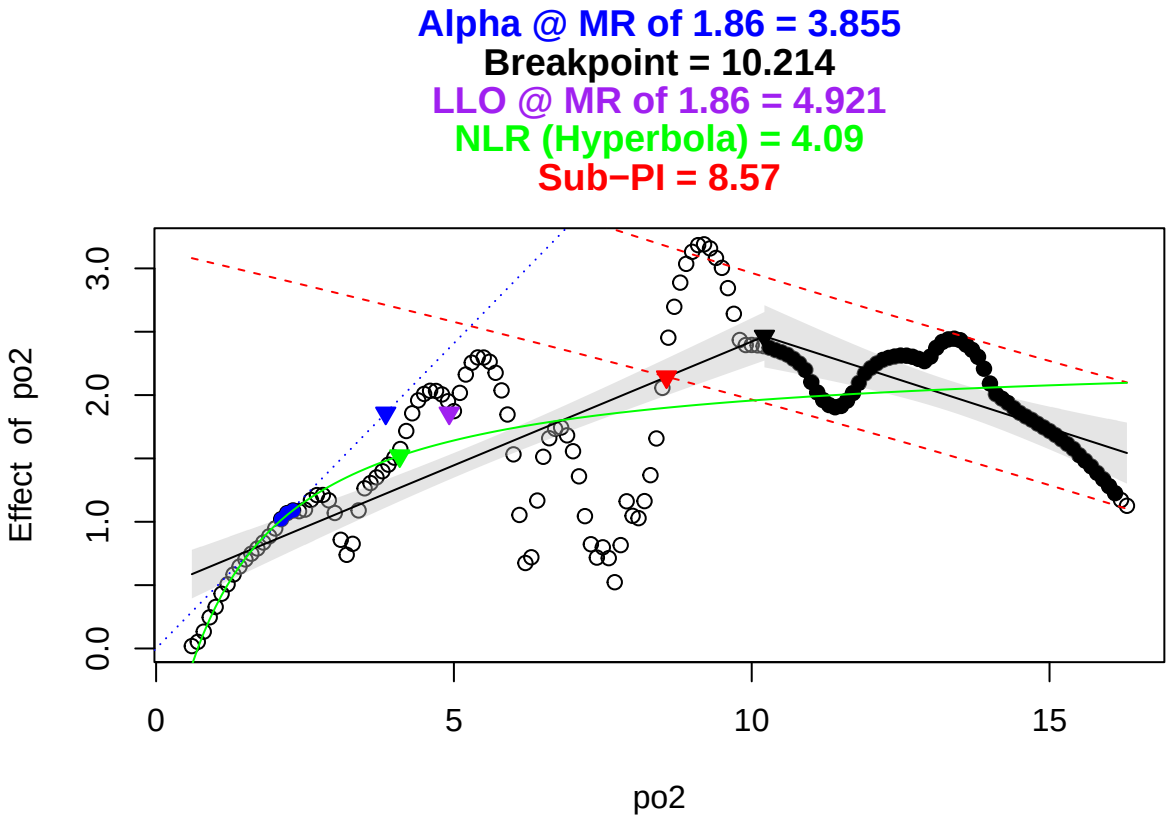

gr2muus1800−2 pcrit in 25 ml jar 7−29−21 ch2 is blank.txt
mass= 2.6
resp vol=0.025
drop time=1.2 hr
O2 drop=253.9
rough mean MO2=2
pcrit.raw[, 3 + ch]
50
100
200
0
1000
2000
3000
4000
5000
6000
7000
pcrit.raw$times

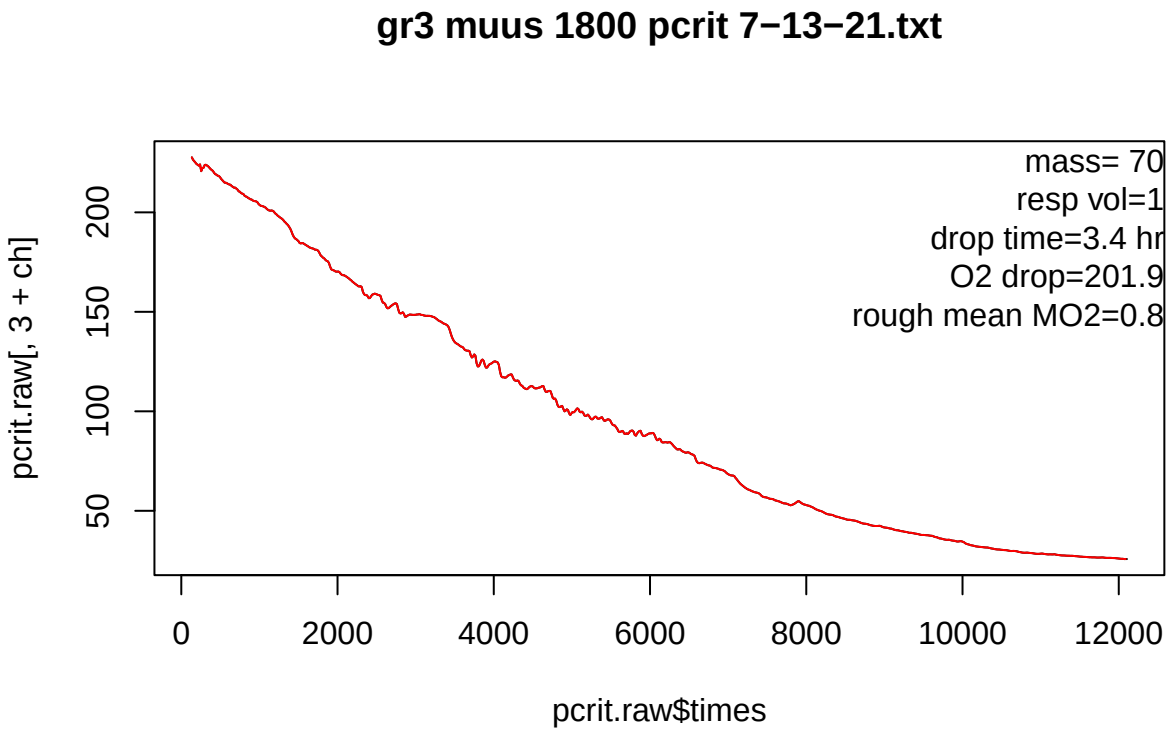

Alpha @ MR of 3.66 = 4.289
Breakpoint = 9.8
LLO @ MR of NA = NA
NLR (Power) = 40.873
Sub−PI = 3.21
Effect of po2
1
2
3
4
5
10
15
20
po2

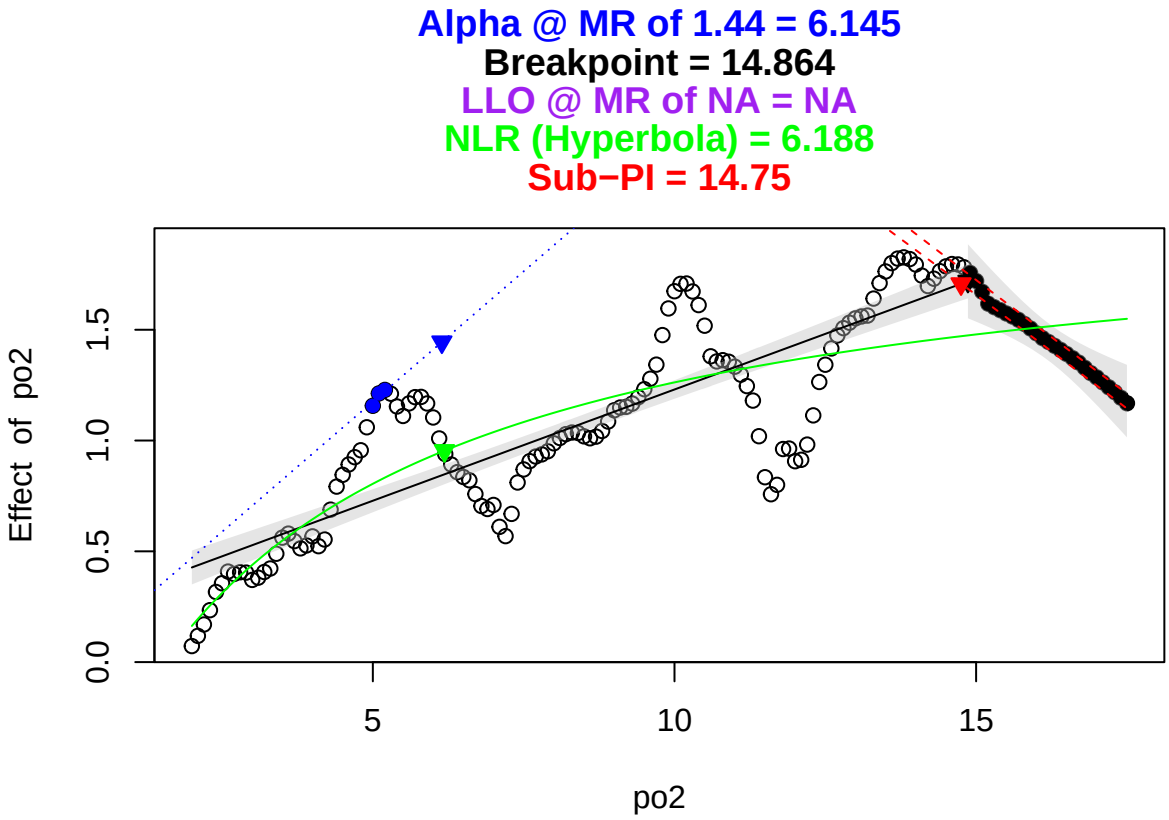

Gr3 Muus 1000 pcrit 7−21−21.txt
mass= 21.6
resp vol=1
drop time=2.2 hr
O2 drop=238.3
rough mean MO2=5
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
2000
4000
6000
8000
10000
12000
pcrit.raw$times

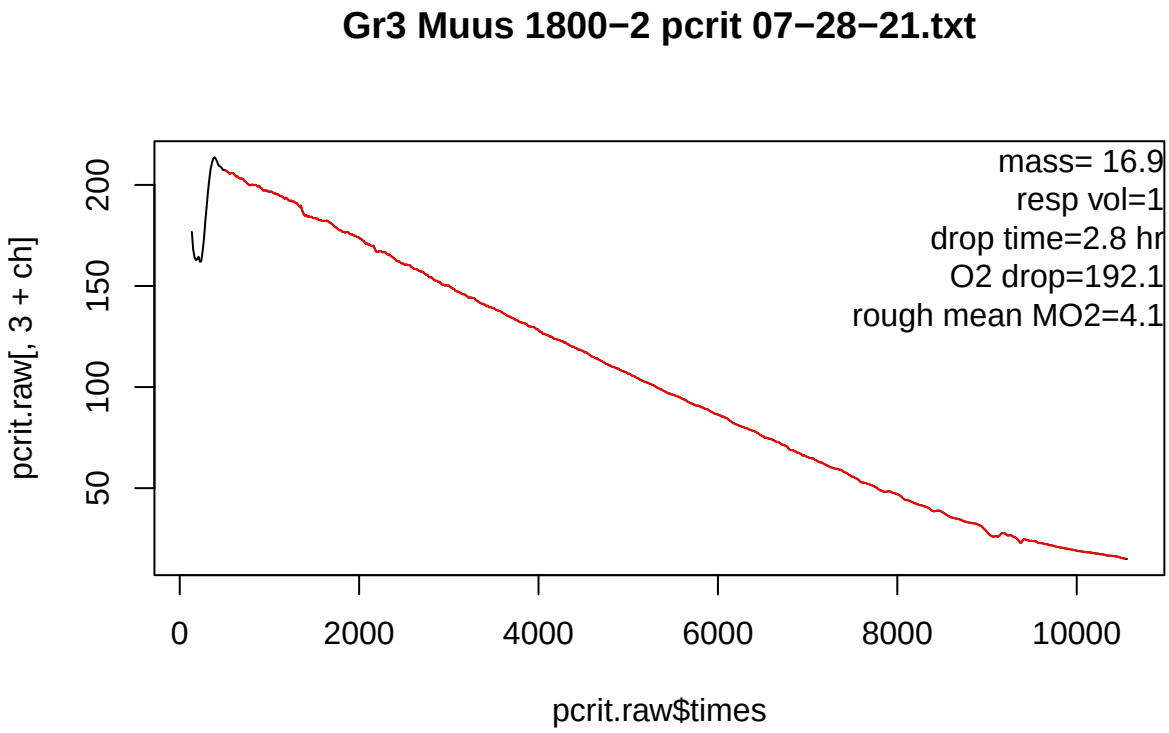

Alpha @ MR of 2.17 = 1.913
Breakpoint = 3.74
LLO @ MR of 2.17 = 1.97
NLR (Power) = 67.77
Sub−PI = 3.68
Effect of po2
2
3
4
5
6
7
5
10
15
po2

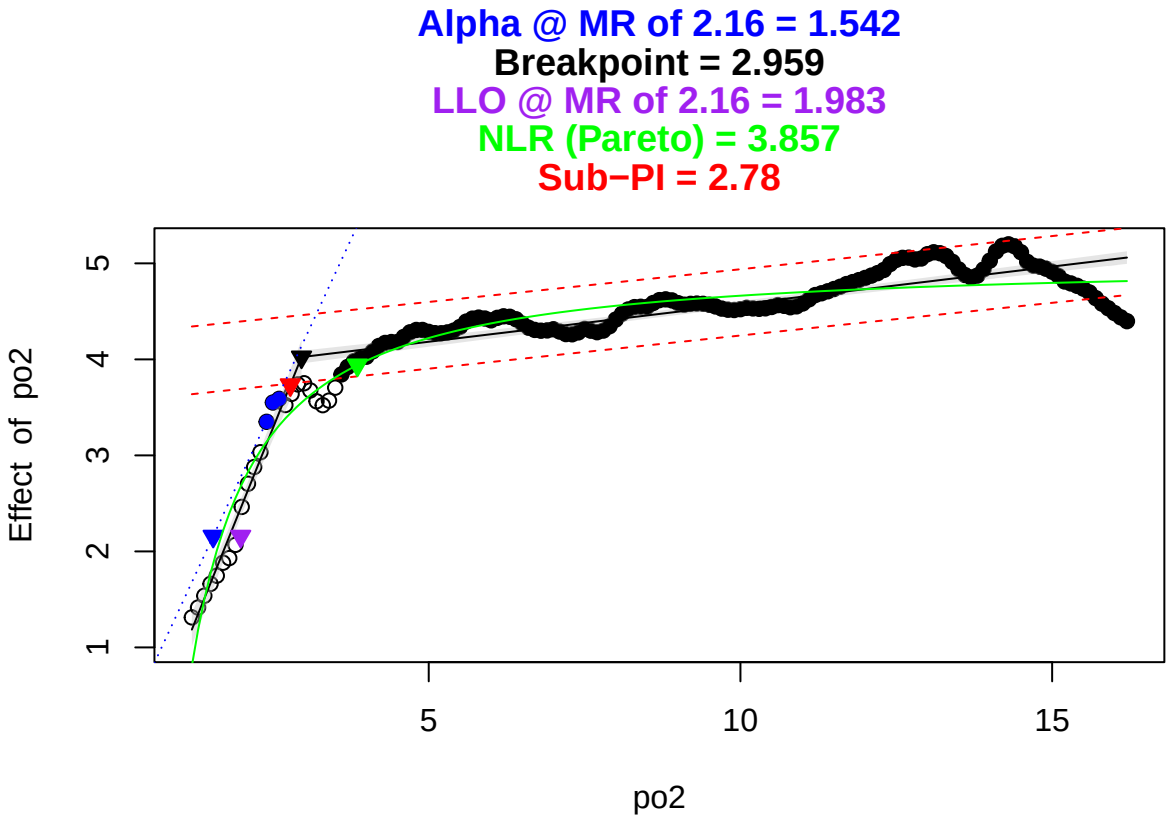

gr3 muus 1800 7day Pcrit 7−20−21.txt
mass= 70
resp vol=1
drop time=2.7 hr
O2 drop=198.3
rough mean MO2=1
pcrit.raw[, 3 + ch]
0
50
100
150
200
0
2000
4000
6000
8000
10000
pcrit.raw$times

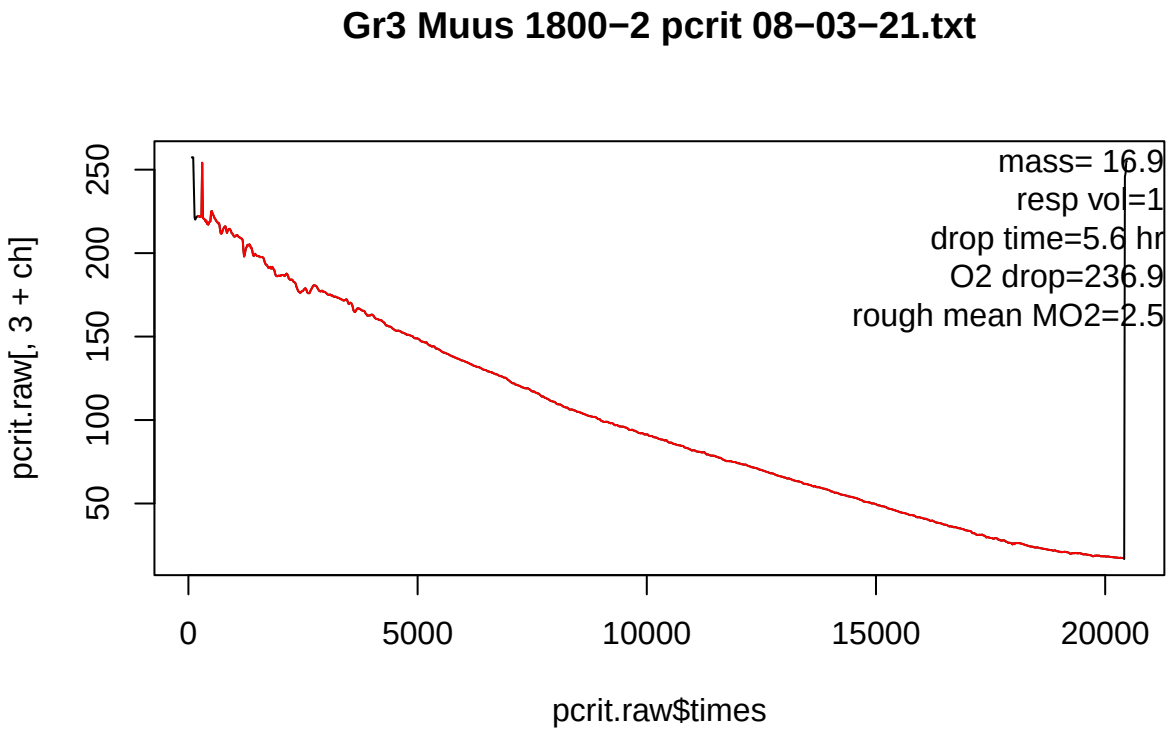

Alpha @ MR of 1.86 = 3.855
Breakpoint = 10.214
LLO @ MR of 1.86 = 4.921
NLR (Hyperbola) = 4.09
Sub−PI = 8.57
Effect of po2
0.0
1.0
2.0
3.0
0
5
10
15
po2

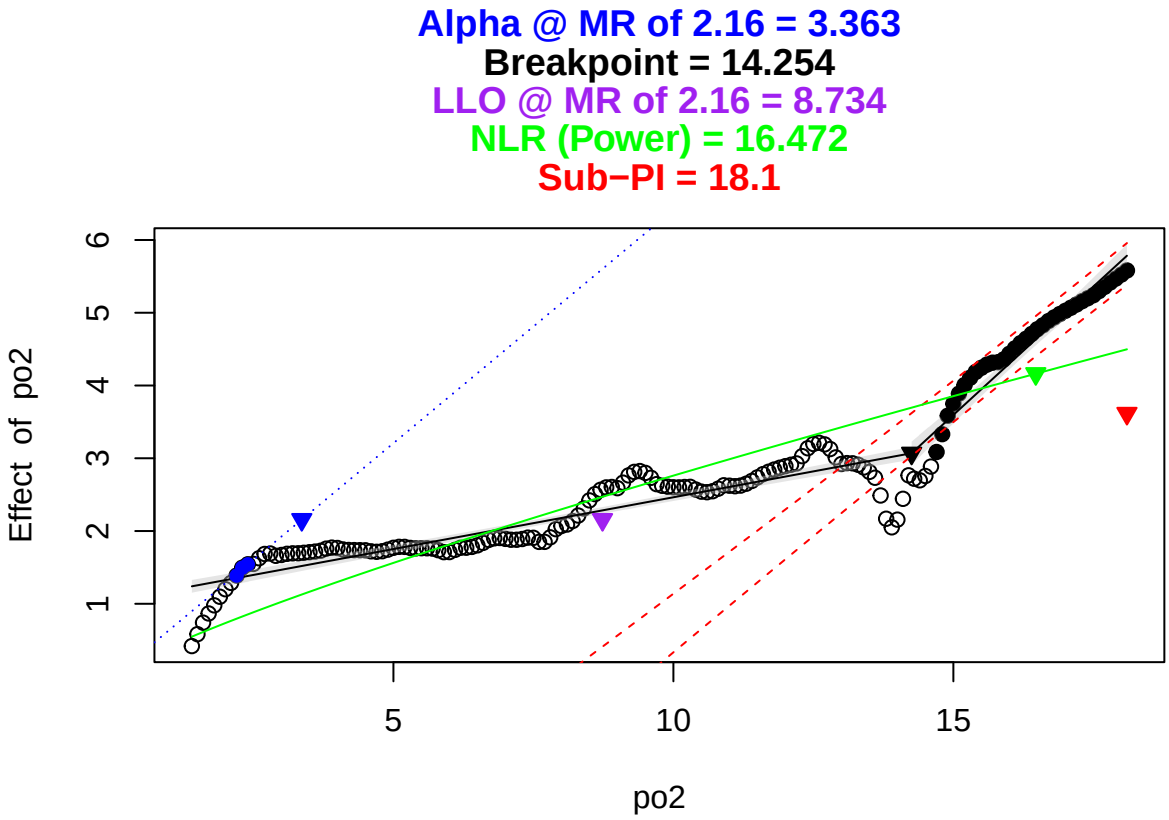

gr3 muus 1800 pcrit 7−13−21.txt
mass= 70
resp vol=1
drop time=3.4 hr
O2 drop=201.9
rough mean MO2=0.8
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
10000
12000
pcrit.raw$times

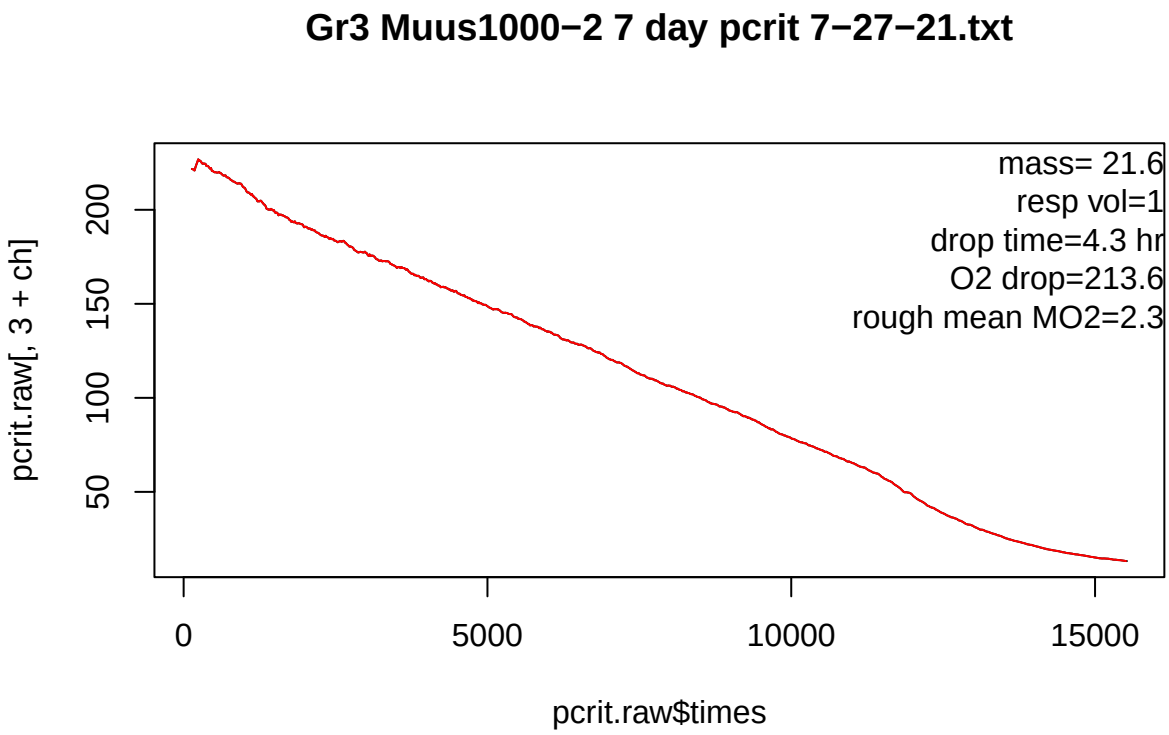

Alpha @ MR of 1.44 = 6.145
Breakpoint = 14.864
LLO @ MR of NA = NA
NLR (Hyperbola) = 6.188
Sub−PI = 14.75
Effect of po2
0.0
0.5
1.0
1.5
5
10
15
po2

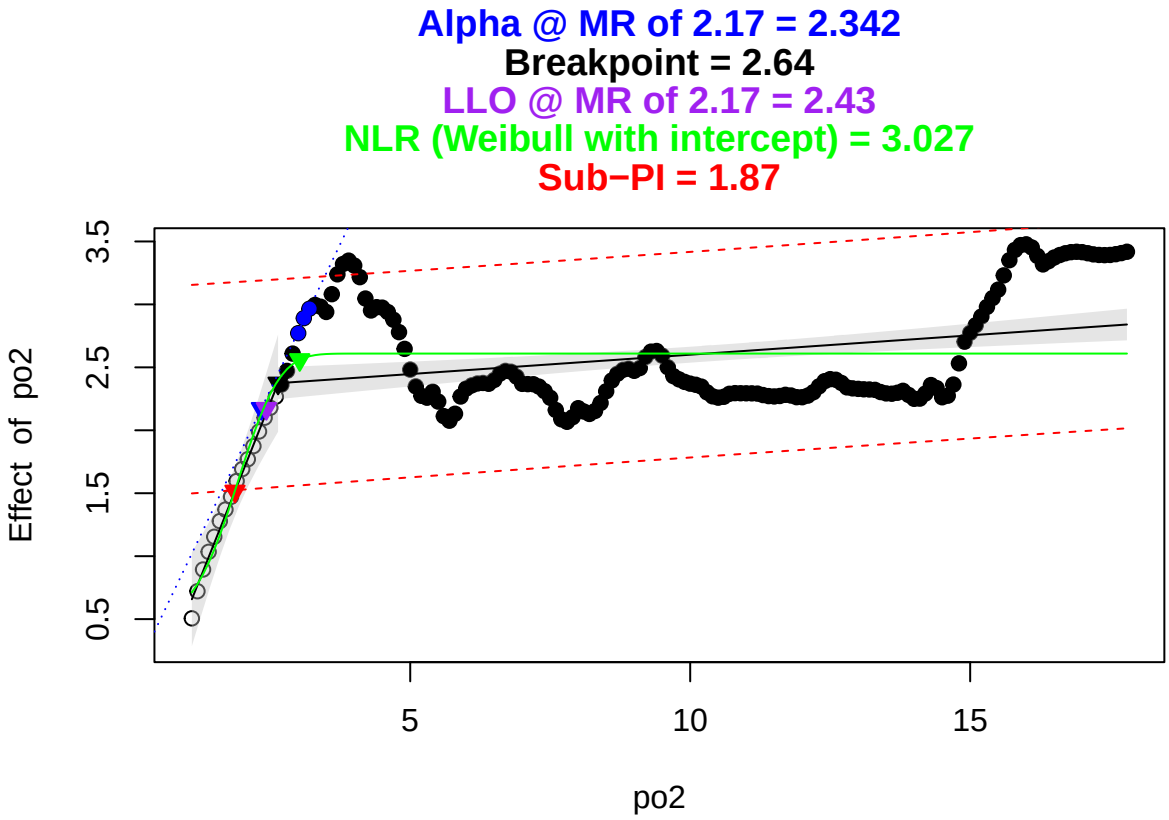

Gr3 Muus 1800−2 pcrit 07−28−21.txt
mass= 16.9
resp vol=1
drop time=2.8 hr
O2 drop=192.1
rough mean MO2=4.1
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
10000
pcrit.raw$times

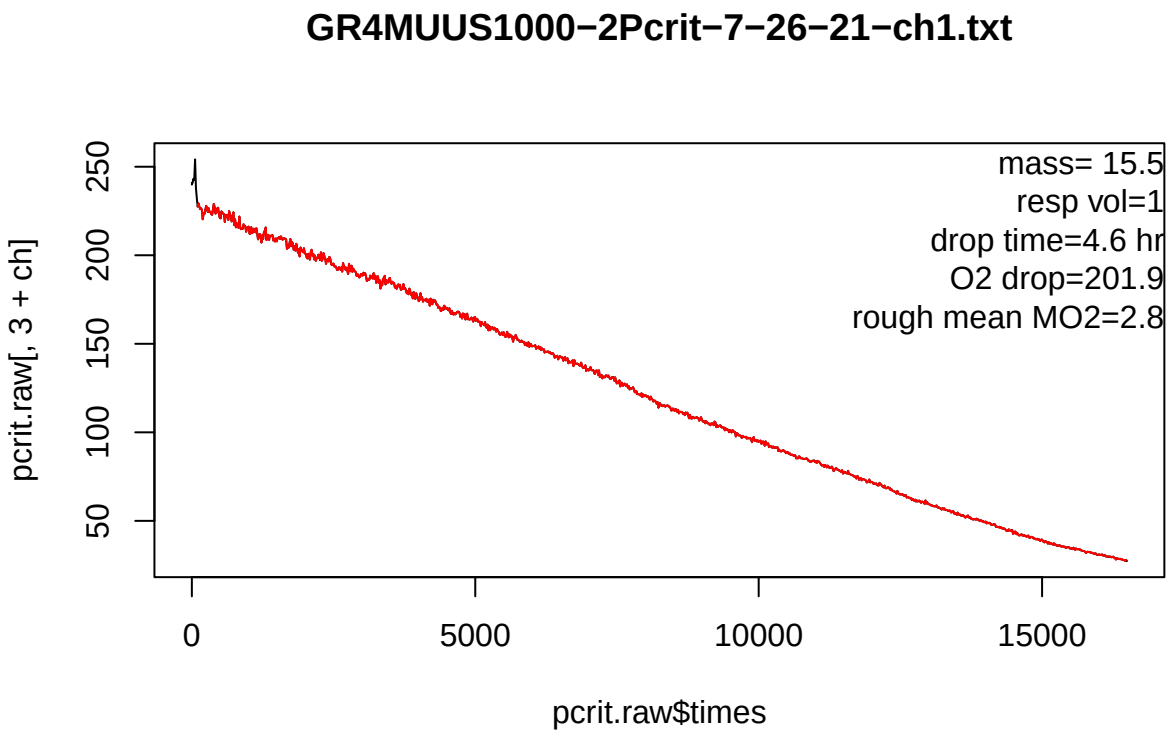

Alpha @ MR of 2.16 = 1.542
Breakpoint = 2.959
LLO @ MR of 2.16 = 1.983
NLR (Pareto) = 3.857
Sub−PI = 2.78
Effect of po2
1
2
3
4
5
5
10
15
po2

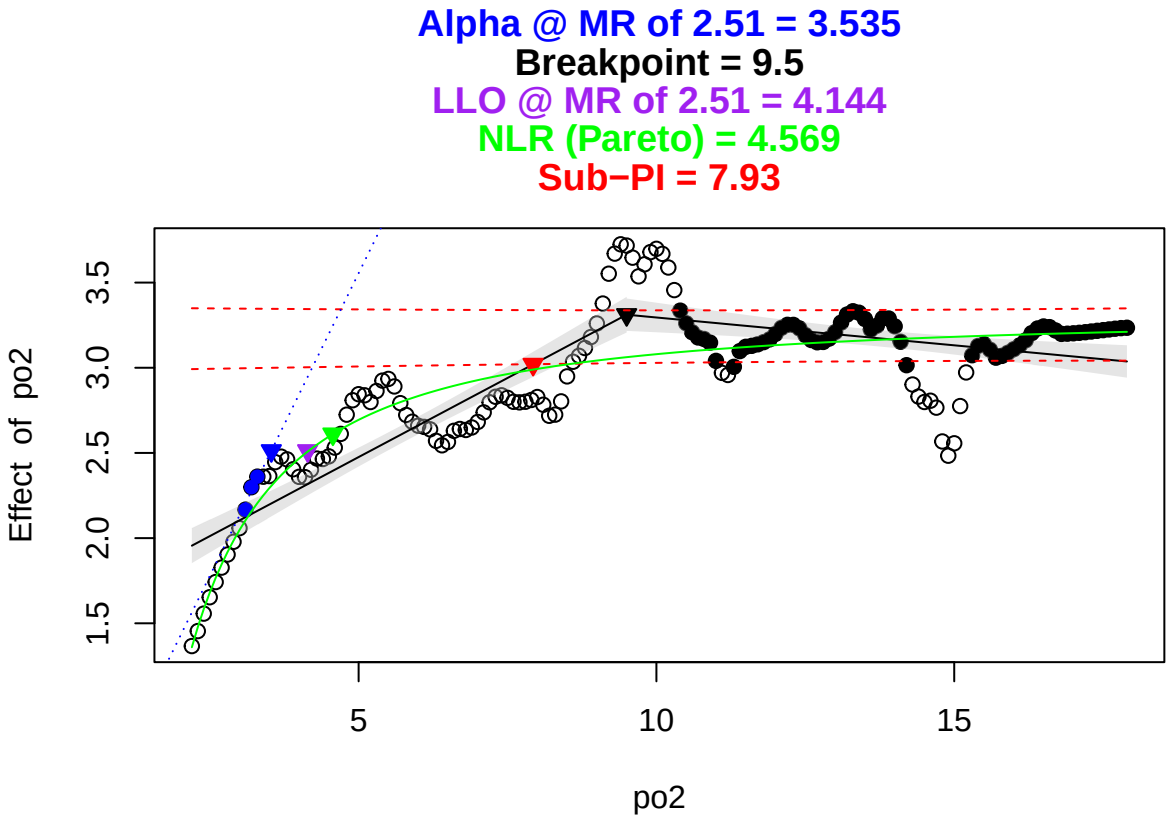

Gr3 Muus 1800−2 pcrit 08−03−21.txt
mass= 16.9
resp vol=1
drop time=5.6 hr
O2 drop=236.9
rough mean MO2=2.5
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
20000
pcrit.raw$times

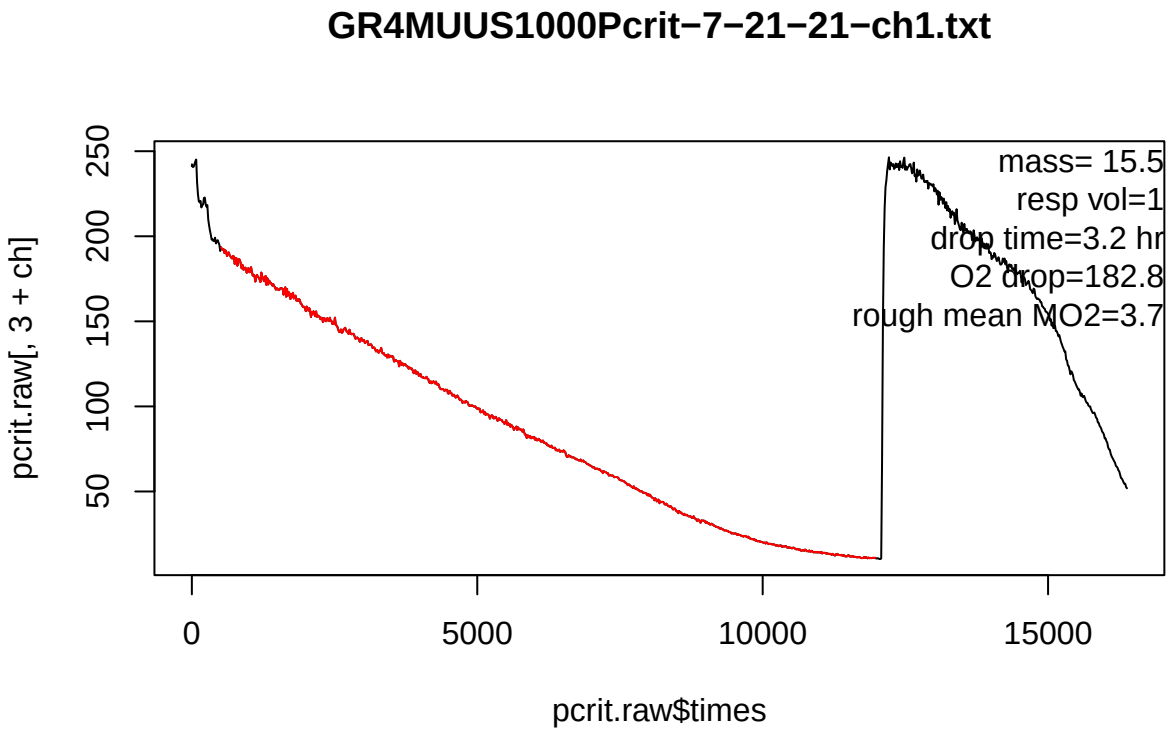

Alpha @ MR of 2.16 = 3.363
Breakpoint = 14.254
LLO @ MR of 2.16 = 8.734
NLR (Power) = 16.472
Sub−PI = 18.1
Effect of po2
1
2
3
4
5
6
5
10
15
po2

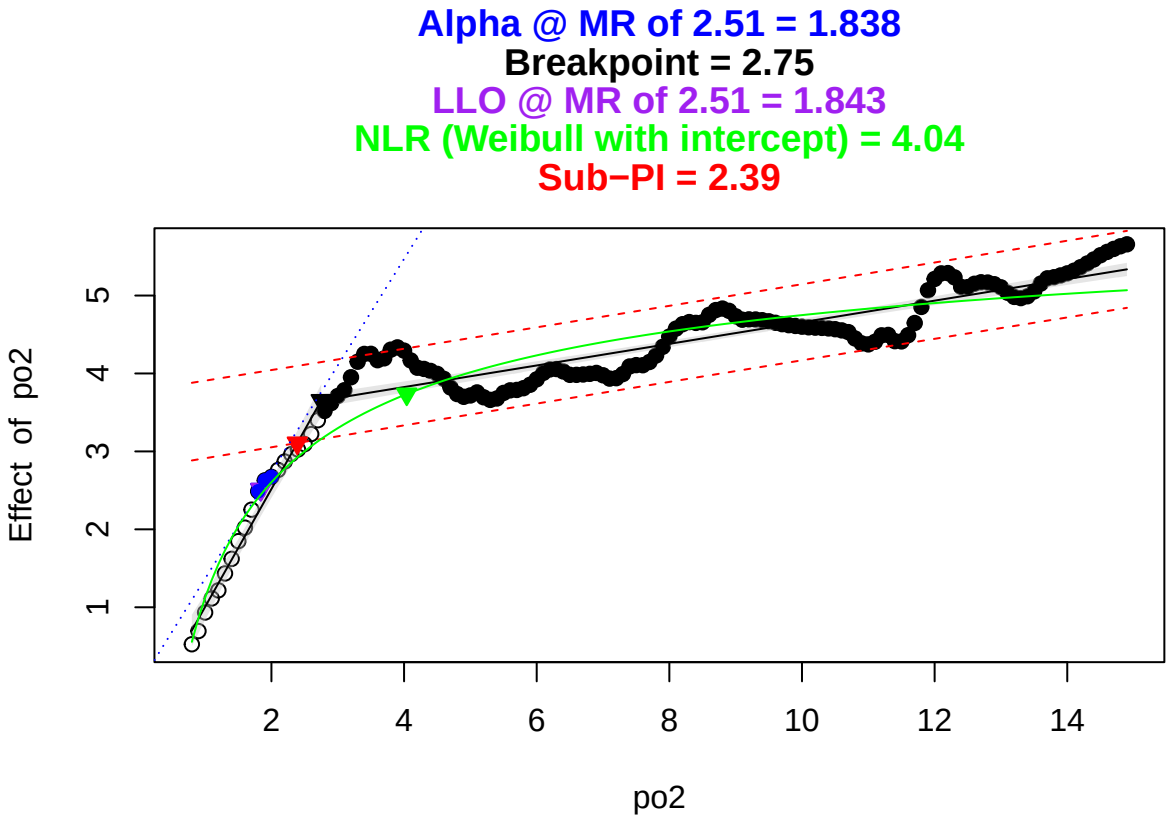

Gr3 Muus1000−2 7 day pcrit 7−27−21.txt
mass= 21.6
resp vol=1
drop time=4.3 hr
O2 drop=213.6
rough mean MO2=2.3
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
pcrit.raw$times

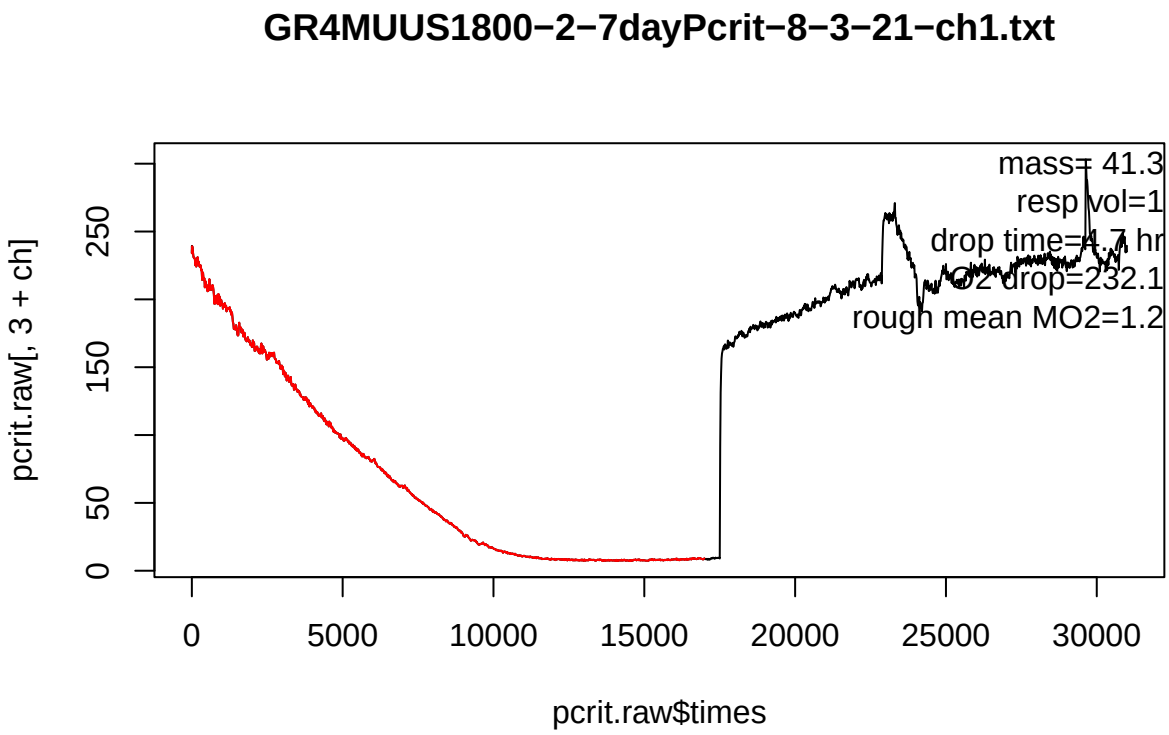

Alpha @ MR of 2.17 = 2.342
Breakpoint = 2.64
LLO @ MR of 2.17 = 2.43
NLR (Weibull with intercept) = 3.027
Sub−PI = 1.87
Effect of po2
0.5
1.5
2.5
3.5
5
10
15
po2

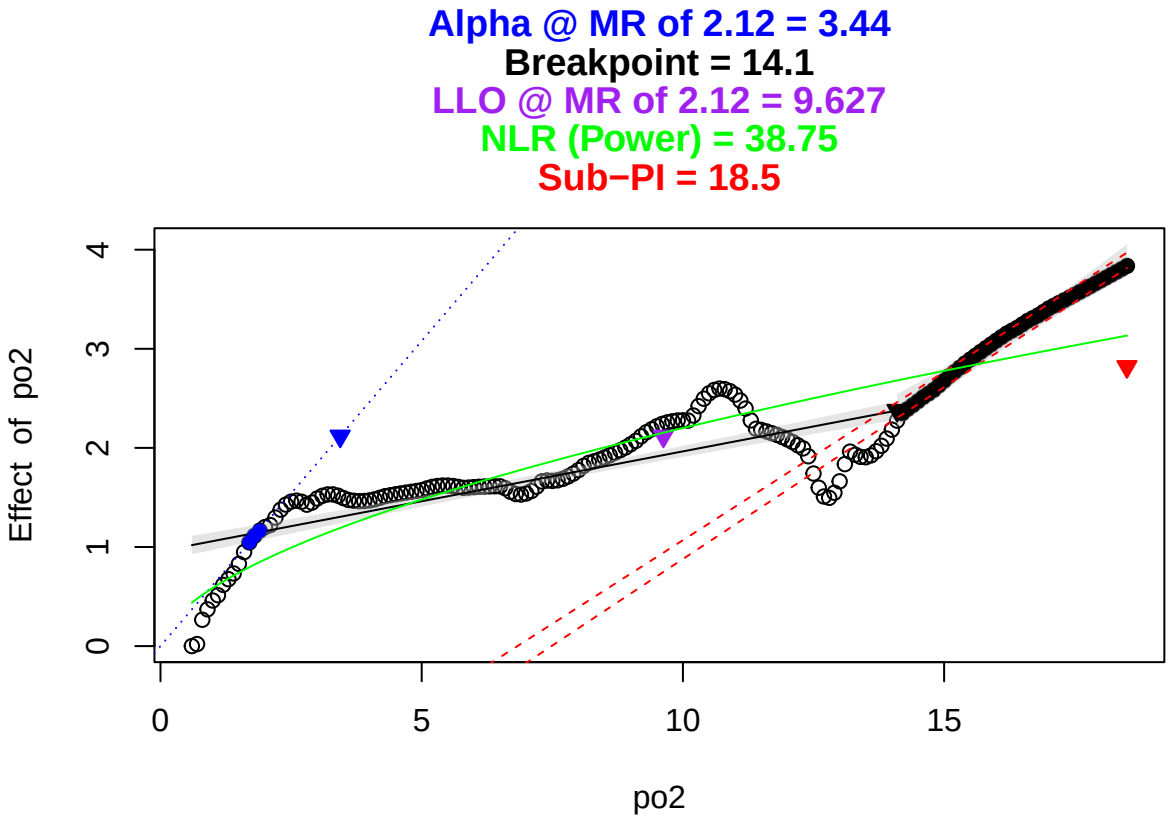

GR4MUUS1000−2Pcrit−7−26−21−ch1.txt
mass= 15.5
resp vol=1
drop time=4.6 hr
O2 drop=201.9
rough mean MO2=2.8
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
pcrit.raw$times

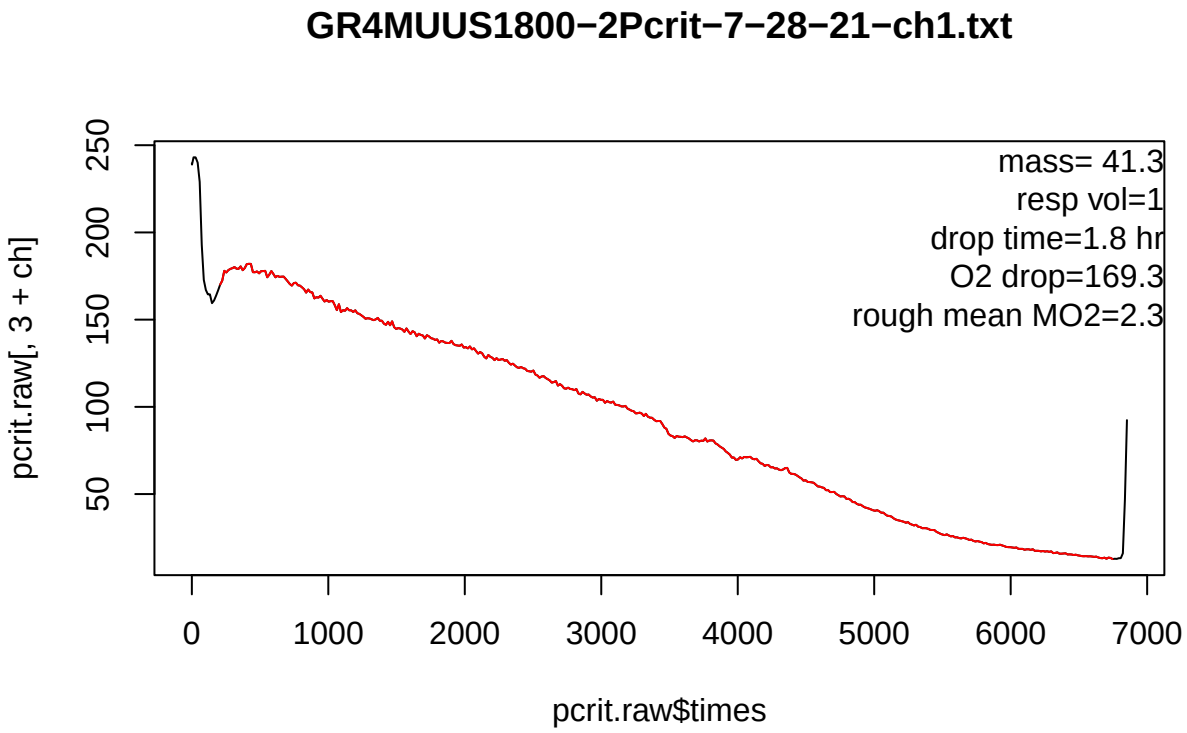

Alpha @ MR of 2.51 = 3.535
Breakpoint = 9.5
LLO @ MR of 2.51 = 4.144
NLR (Pareto) = 4.569
Sub−PI = 7.93
Effect of po2
1.5
2.0
2.5
3.0
3.5
5
10
15
po2

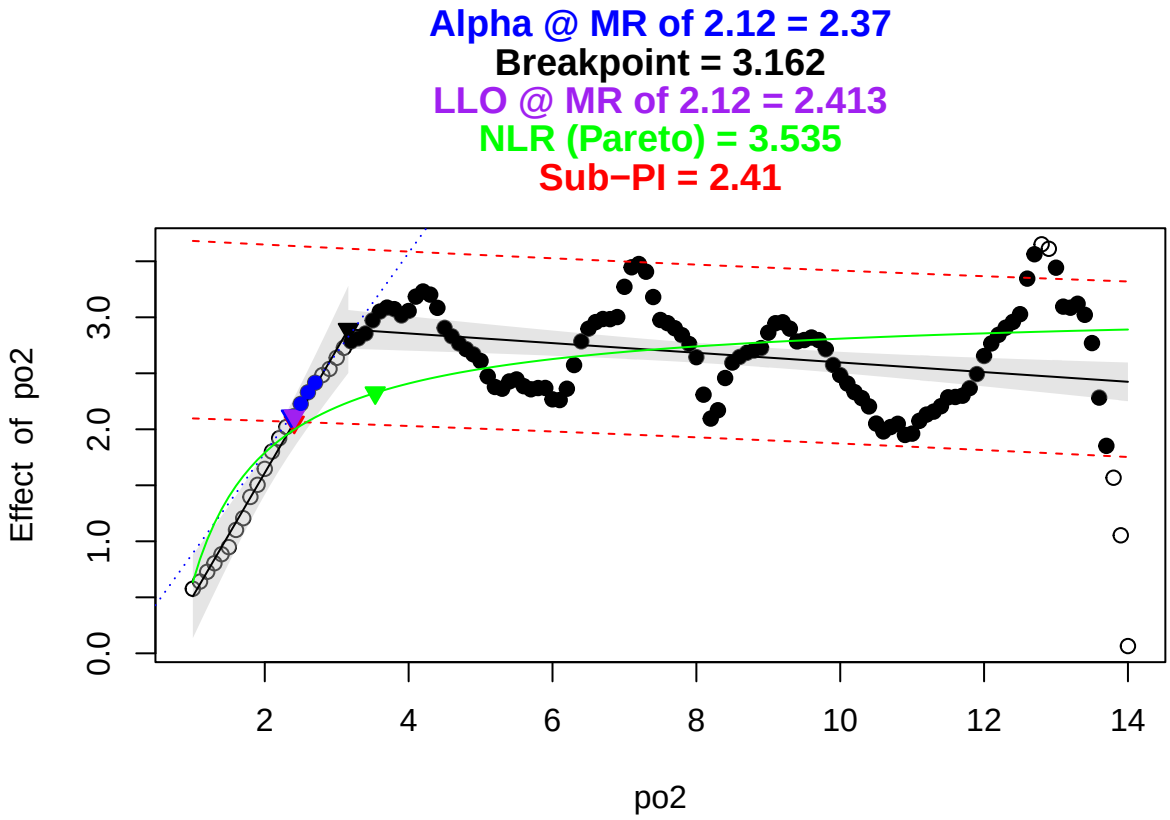

GR4MUUS1000Pcrit−7−21−21−ch1.txt
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
pcrit.raw$times
mass= 15.5
resp vol=1
drop time=3.2 hr
O2 drop=182.8
rough mean MO2=3.7

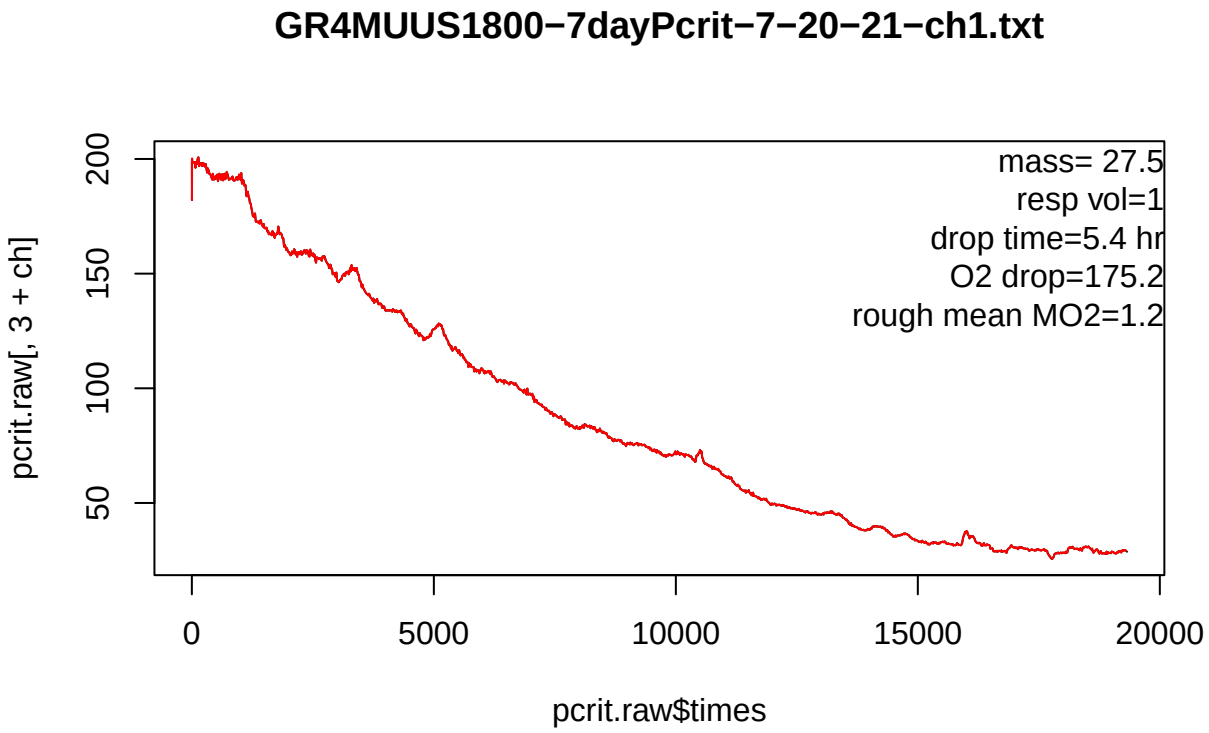

Alpha @ MR of 2.51 = 1.838
Breakpoint = 2.75
LLO @ MR of 2.51 = 1.843
NLR (Weibull with intercept) = 4.04
Sub−PI = 2.39
Effect of po2
1
2
3
4
5
2
4
6
8
10
12
14
po2

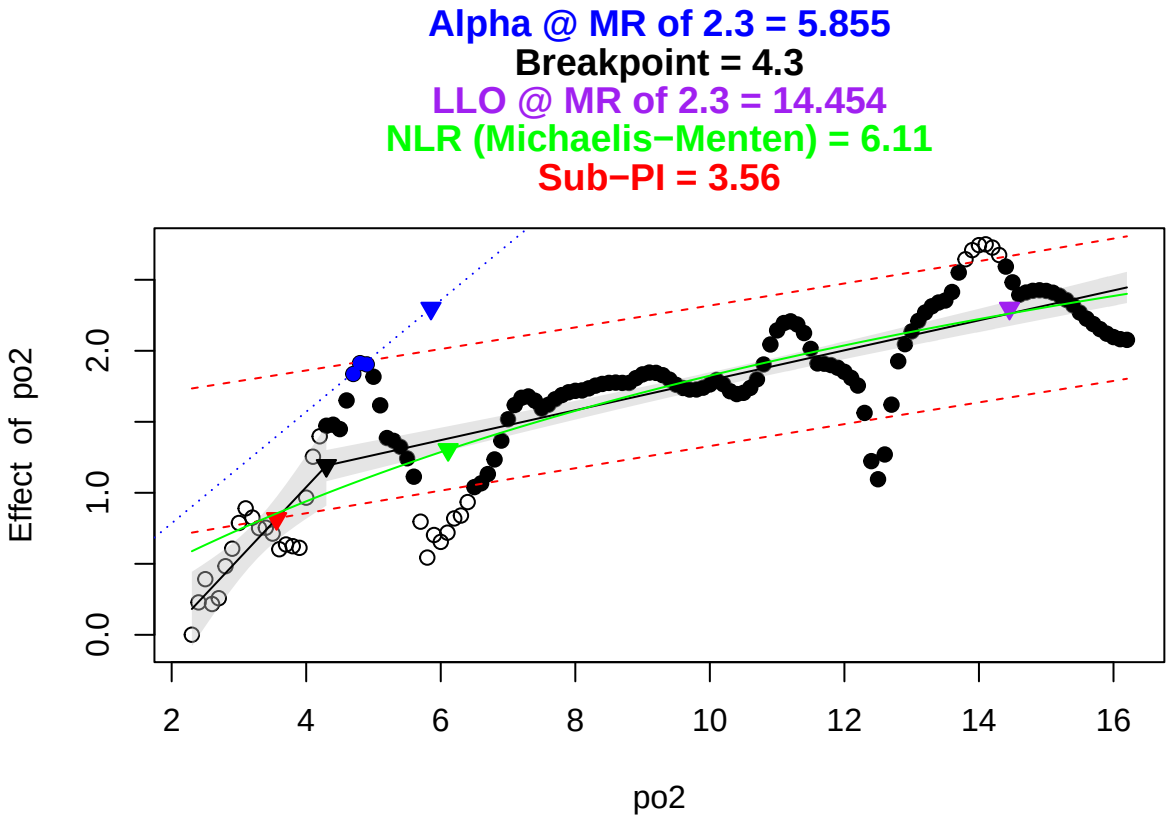

GR4MUUS1800−2−7dayPcrit−8−3−21−ch1.txt
mass= 41.3
resp vol=1
drop time=4.7 hr
O2 drop=232.1
rough mean MO2=1.2
pcrit.raw[, 3 + ch]
0
50
150
250
0
5000
10000
15000
20000
25000
30000
pcrit.raw$times

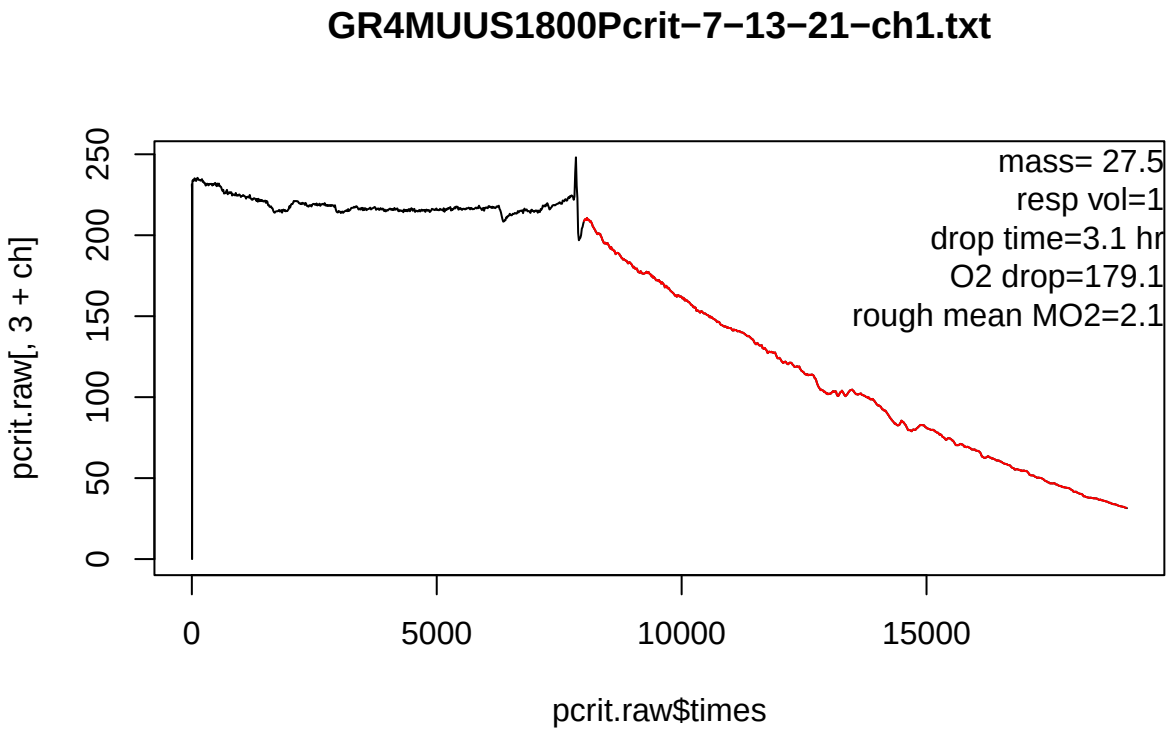

Alpha @ MR of 2.12 = 3.44
Breakpoint = 14.1
LLO @ MR of 2.12 = 9.627
NLR (Power) = 38.75
Sub−PI = 18.5
Effect of po2
0
1
2
3
4
0
5
10
15
po2

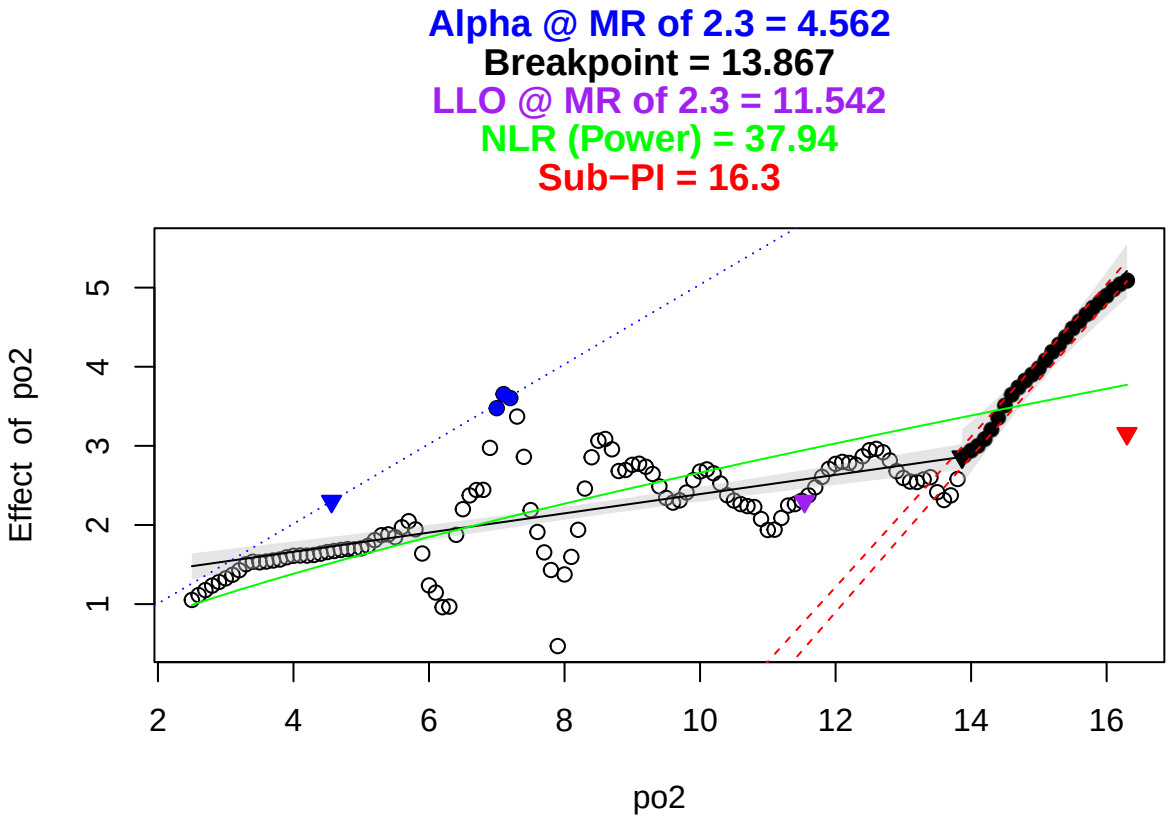

GR4MUUS1800−2Pcrit−7−28−21−ch1.txt
mass= 41.3
resp vol=1
drop time=1.8 hr
O2 drop=169.3
rough mean MO2=2.3
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
1000
2000
3000
4000
5000
6000
7000
pcrit.raw$times

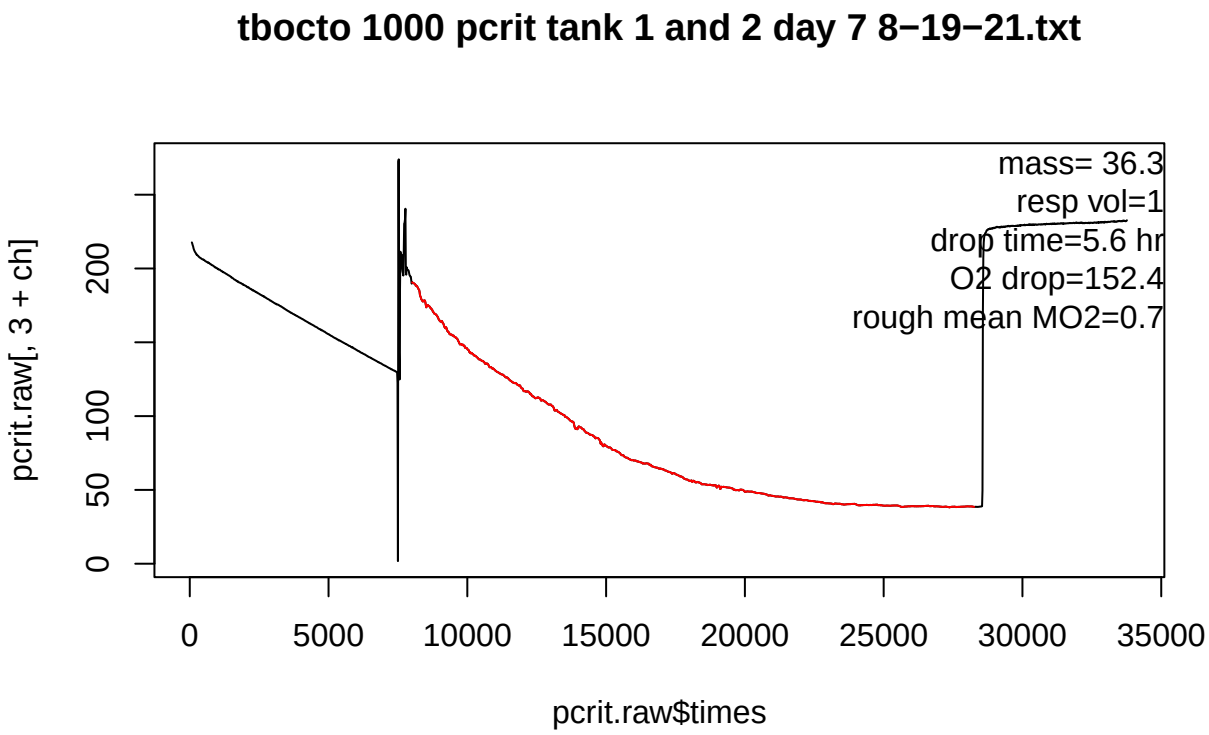

Alpha @ MR of 2.12 = 2.37
Breakpoint = 3.162
LLO @ MR of 2.12 = 2.413
NLR (Pareto) = 3.535
Sub−PI = 2.41
Effect of po2
0.0
1.0
2.0
3.0
2
4
6
8
10
12
14
po2

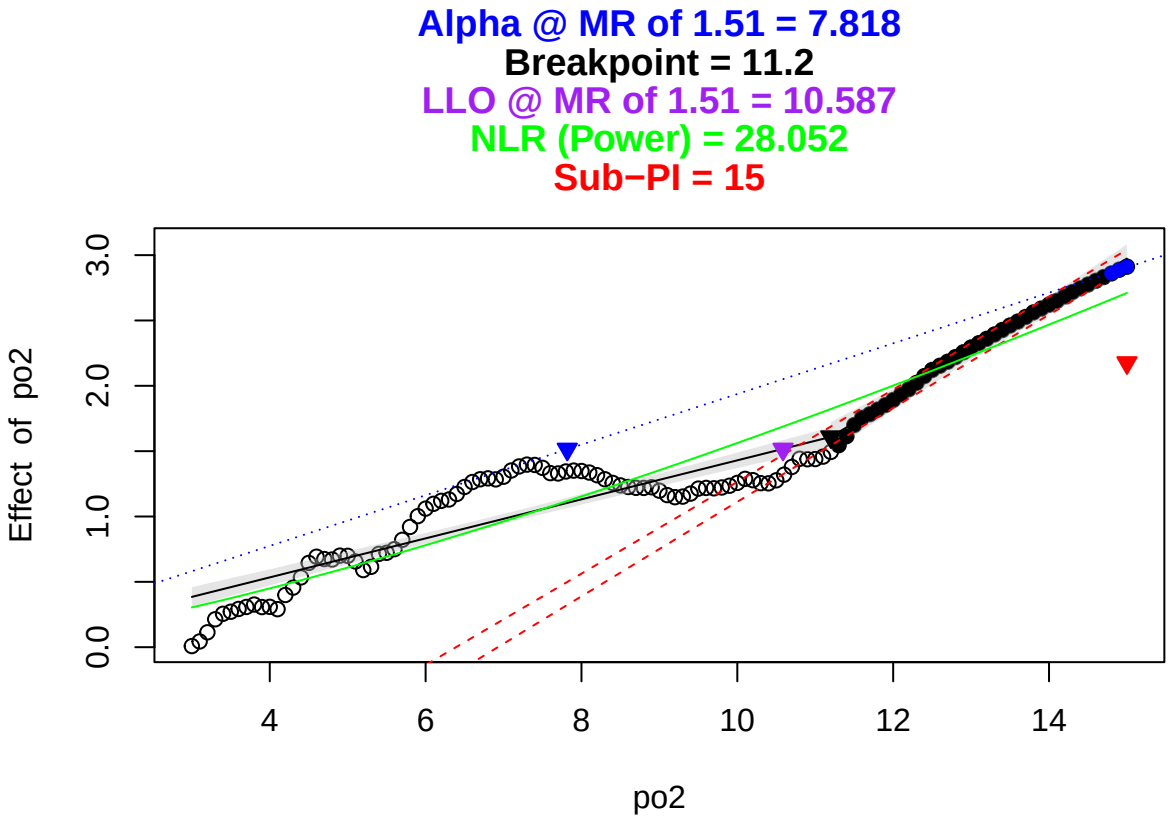

GR4MUUS1800−7dayPcrit−7−20−21−ch1.txt
mass= 27.5
resp vol=1
drop time=5.4 hr
O2 drop=175.2
rough mean MO2=1.2
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
pcrit.raw$times

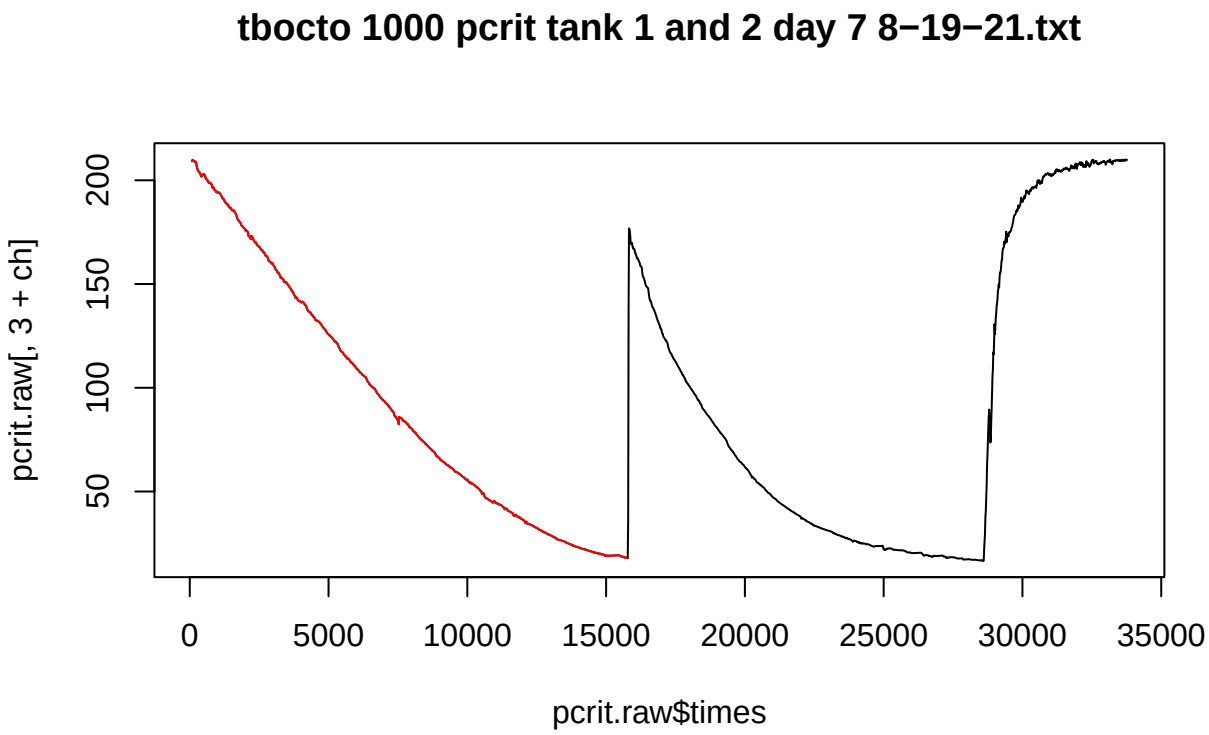

Alpha @ MR of 2.3 = 5.855
Breakpoint = 4.3
LLO @ MR of 2.3 = 14.454
NLR (Michaelis−Menten) = 6.11
Sub−PI = 3.56
Effect of po2
0.0
1.0
2.0
2
4
6
8
10
12
14
16
po2

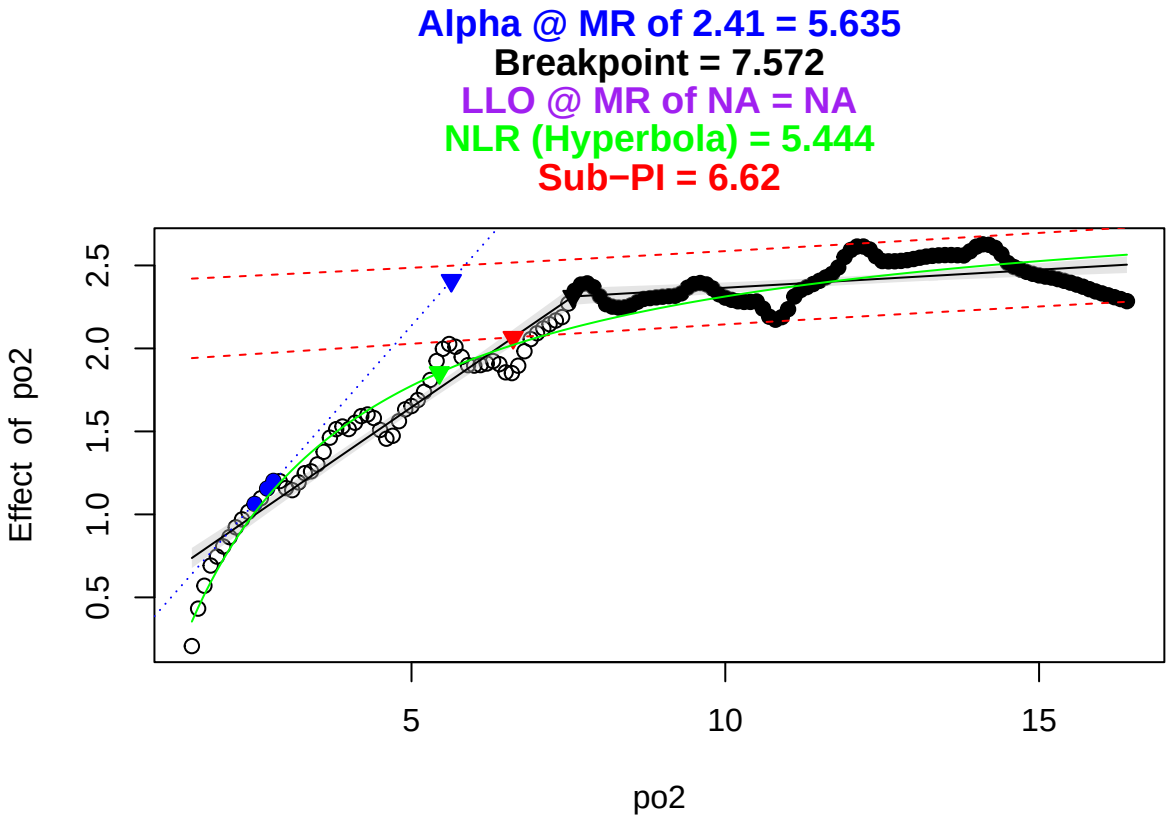

GR4MUUS1800Pcrit−7−13−21−ch1.txt
mass= 27.5
resp vol=1
drop time=3.1 hr
O2 drop=179.1
rough mean MO2=2.1
pcrit.raw[, 3 + ch]
0
50
100
150
200
250
0
5000
10000
15000
pcrit.raw$times

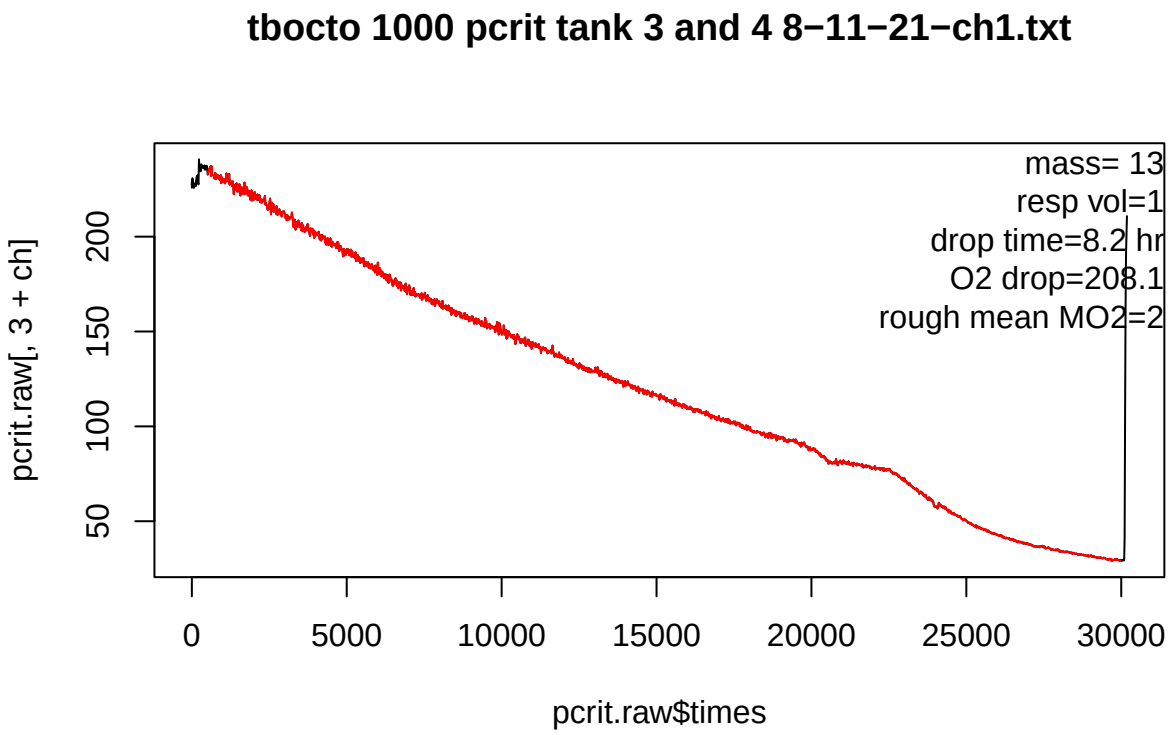

Alpha @ MR of 2.3 = 4.562
Breakpoint = 13.867
LLO @ MR of 2.3 = 11.542
NLR (Power) = 37.94
Sub−PI = 16.3
Effect of po2
po2
1
2
3
4
5
2
4
6
8
10
12
14
16

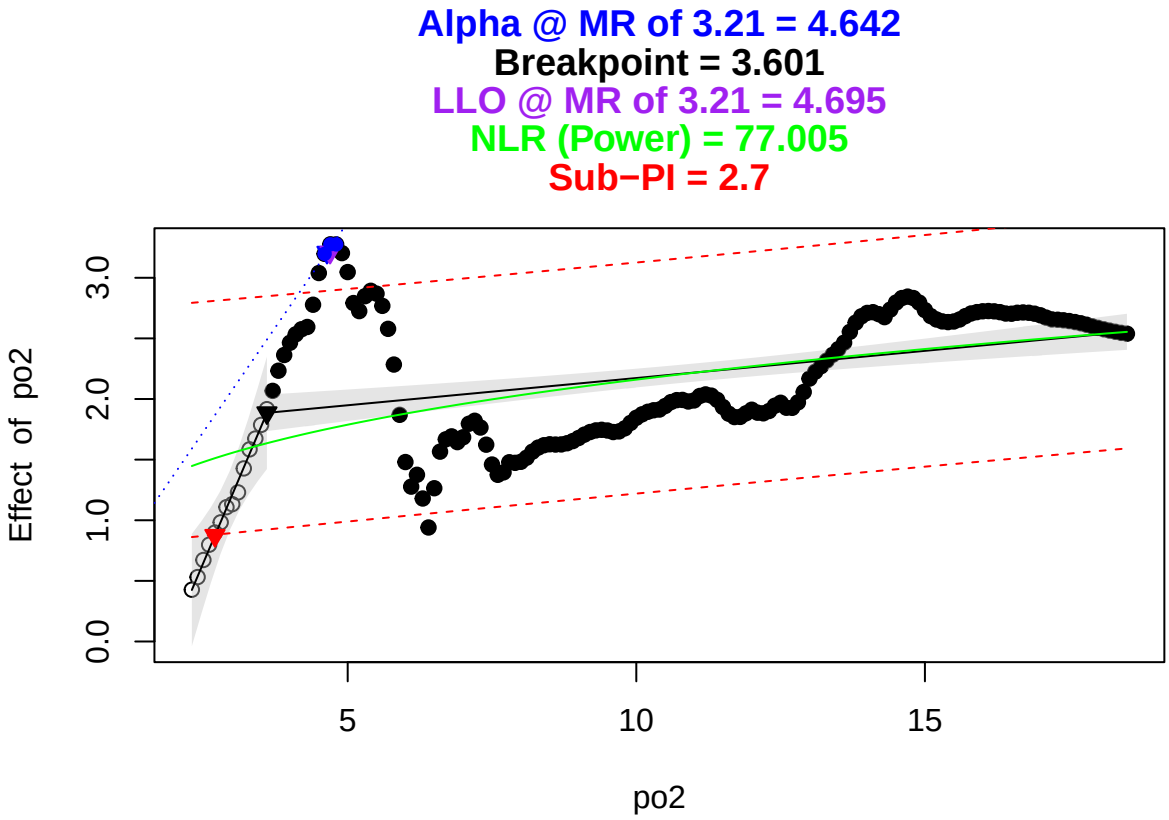

tbocto 1000 pcrit tank 1 and 2 day 7 8−19−21.txt
mass= 36.3
resp vol=1
drop time=5.6 hr
O2 drop=152.4
rough mean MO2=0.7
pcrit.raw[, 3 + ch]
0
50
100
200
0
5000
10000
15000
20000
25000
30000
35000
pcrit.raw$times

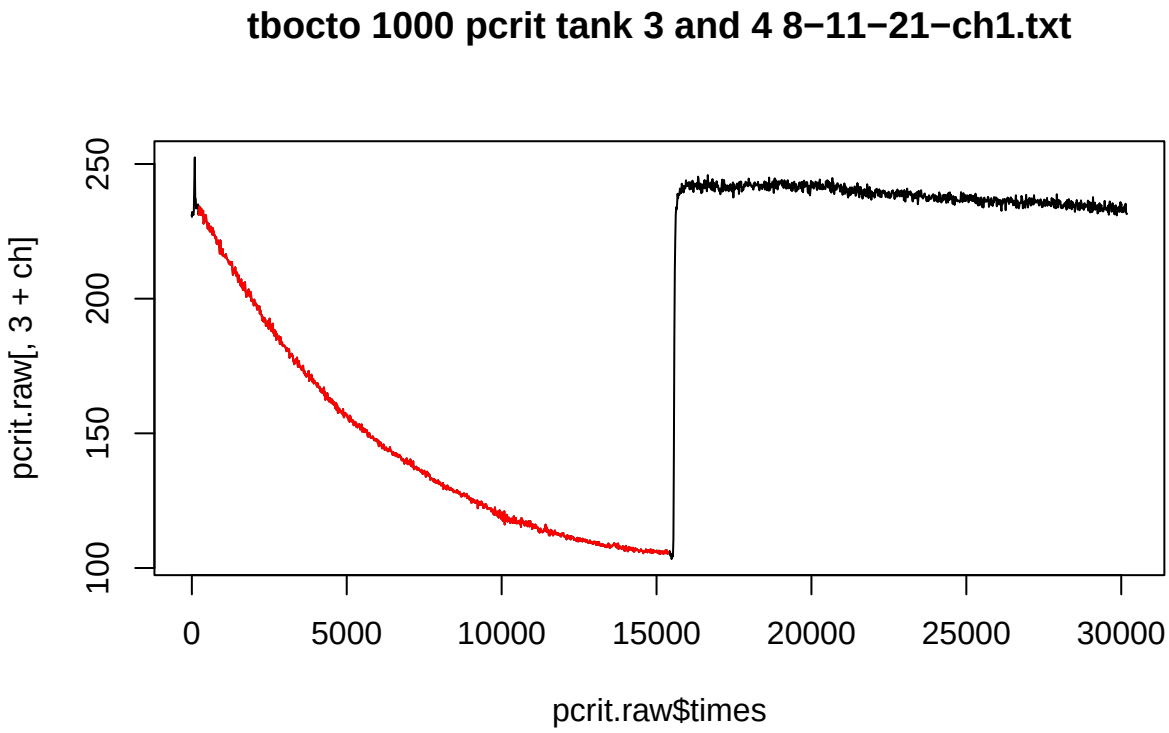

Alpha @ MR of 1.51 = 7.818
Breakpoint = 11.2
LLO @ MR of 1.51 = 10.587
NLR (Power) = 28.052
Sub−PI = 15
Effect of po2
0.0
1.0
2.0
3.0
4
6
8
10
12
14
po2

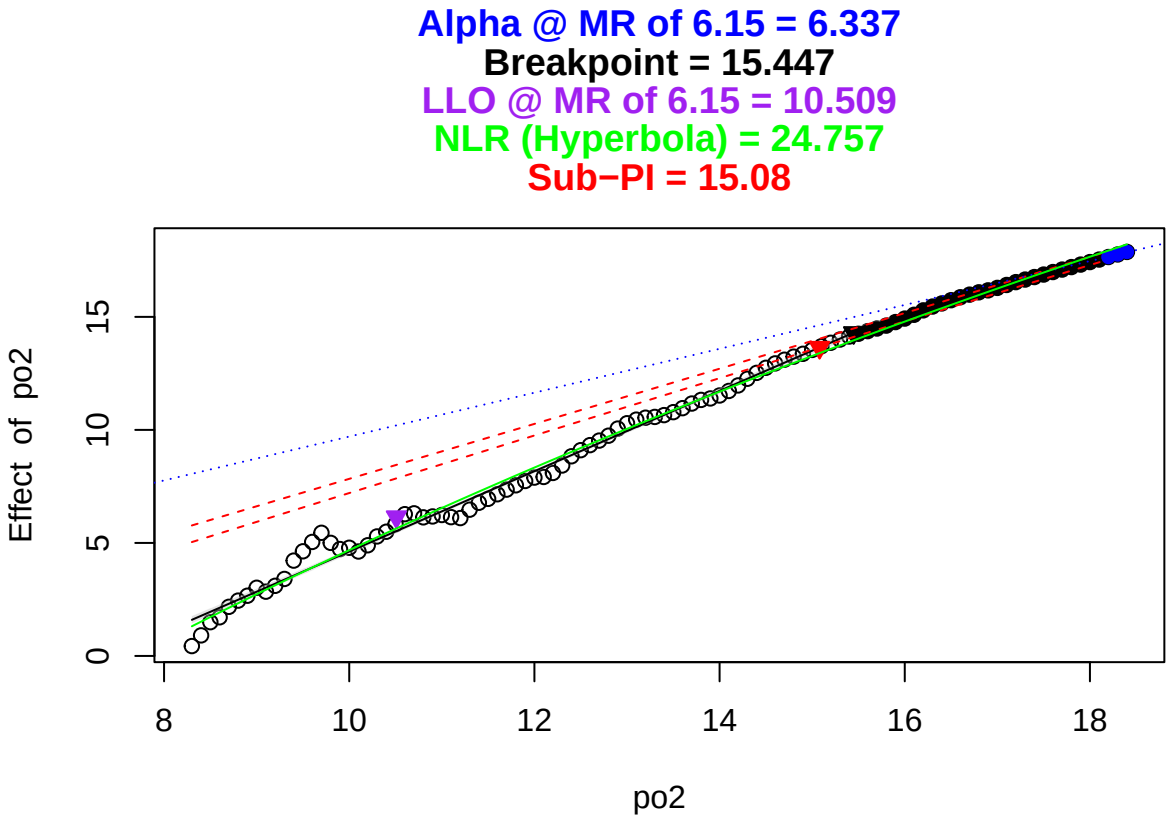

tbocto 1000 pcrit tank 1 and 2 day 7 8−19−21.txt
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
25000
30000
35000
pcrit.raw$times

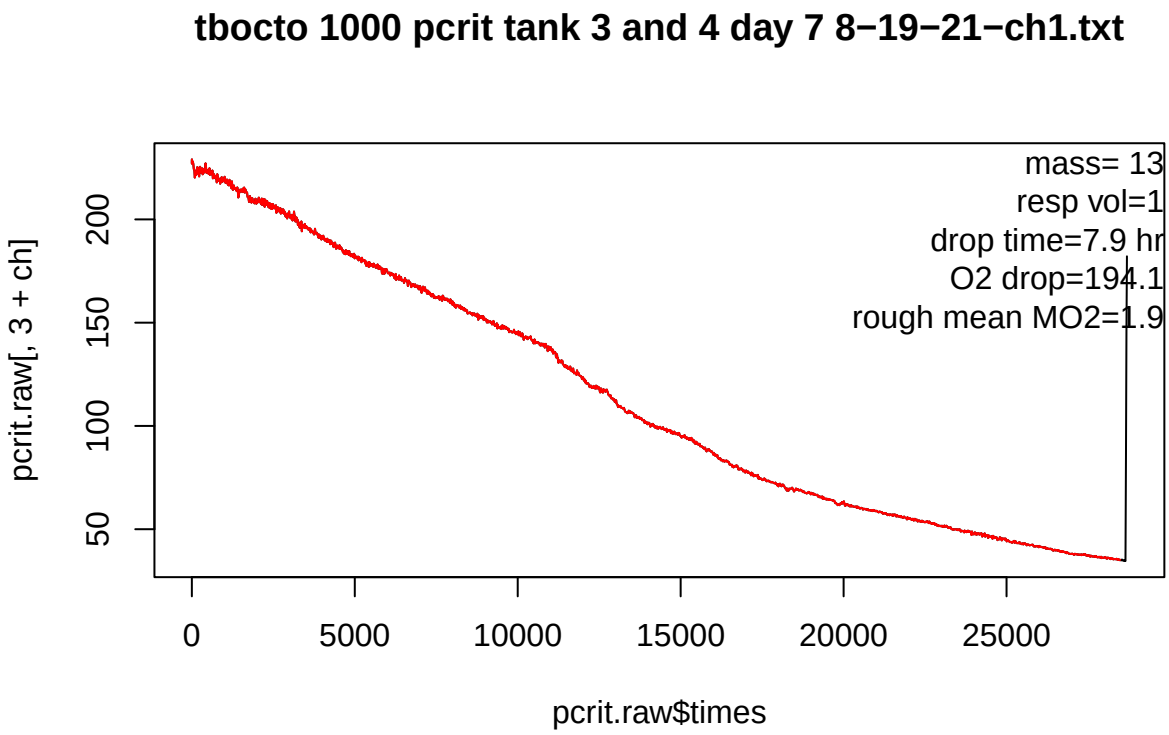

Alpha @ MR of 2.41 = 5.635
Breakpoint = 7.572
LLO @ MR of NA = NA
NLR (Hyperbola) = 5.444
Sub−PI = 6.62
Effect of po2
0.5
1.0
1.5
2.0
2.5
5
10
15
po2

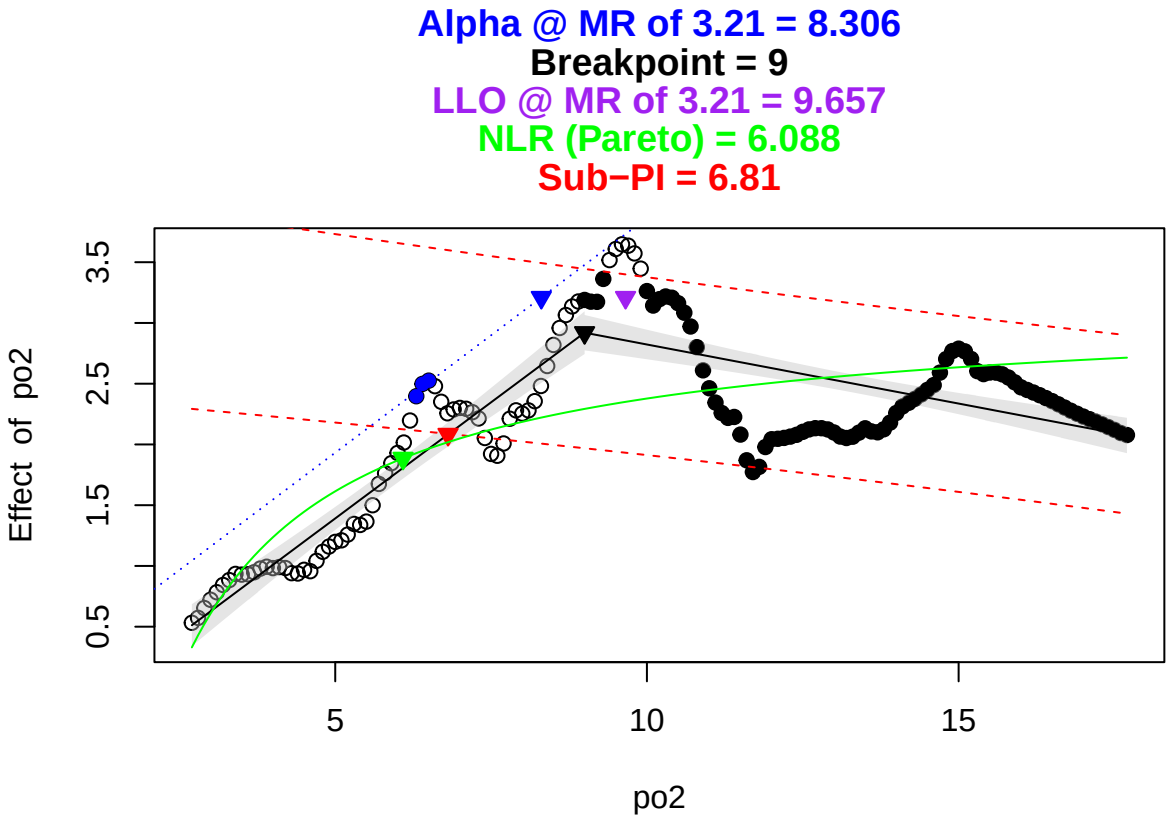

tbocto 1000 pcrit tank 3 and 4 8−11−21−ch1.txt
mass= 13
resp vol=1
drop time=8.2 hr
O2 drop=208.1
rough mean MO2=2
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
25000
30000
pcrit.raw$times

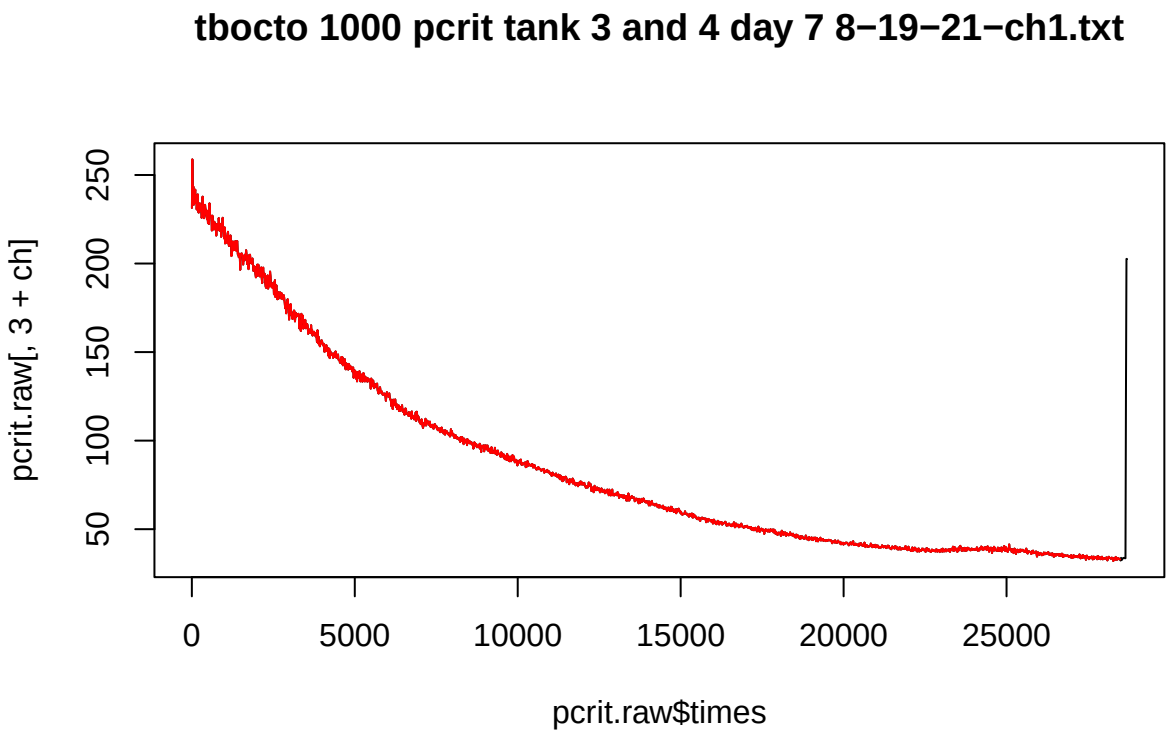

Alpha @ MR of 3.21 = 4.642
Breakpoint = 3.601
LLO @ MR of 3.21 = 4.695
NLR (Power) = 77.005
Sub−PI = 2.7
Effect of po2
0.0
1.0
2.0
3.0
5
10
15
po2

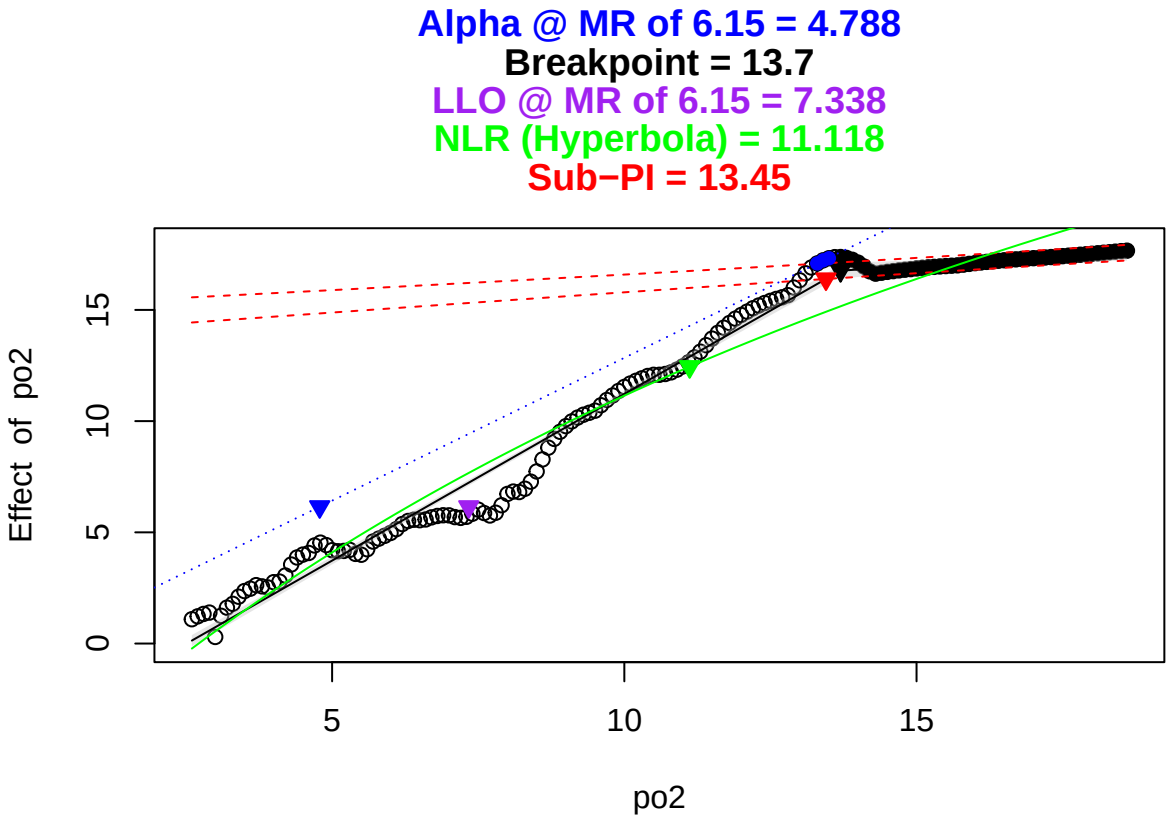

tbocto 1000 pcrit tank 3 and 4 8−11−21−ch1.txt
pcrit.raw[, 3 + ch]
100
150
200
250
0
5000
10000
15000
20000
25000
30000
pcrit.raw$times

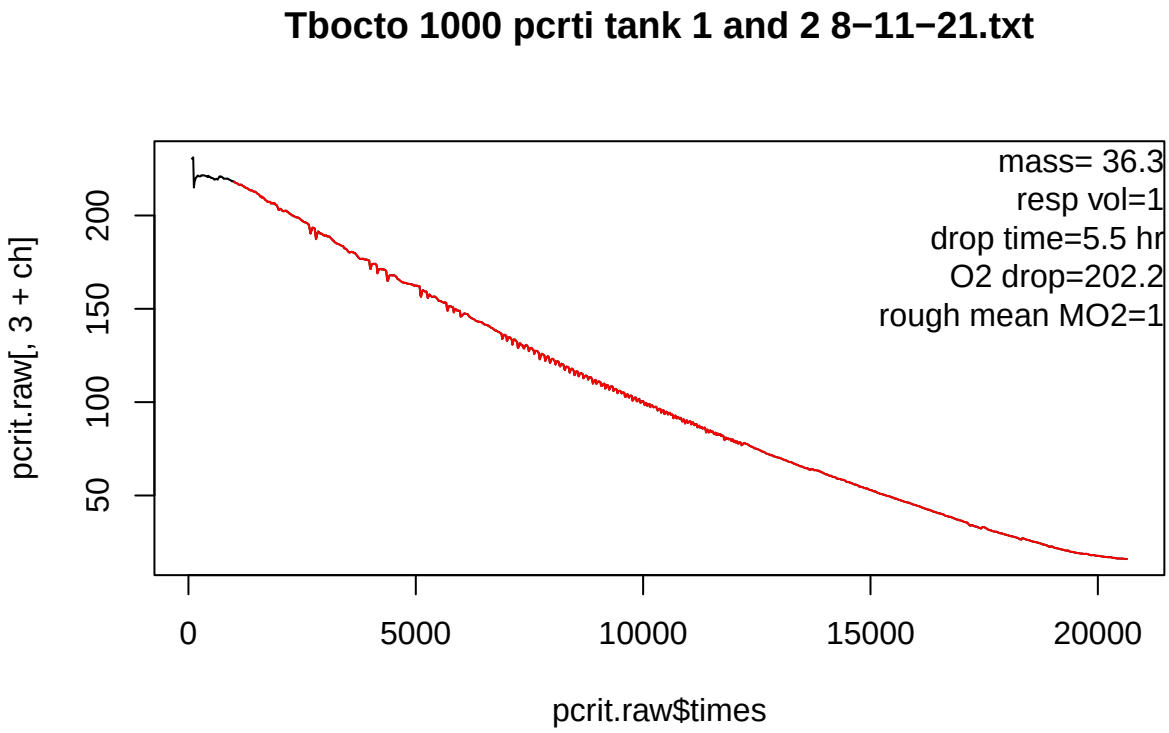

Alpha @ MR of 6.15 = 6.337
Breakpoint = 15.447
LLO @ MR of 6.15 = 10.509
NLR (Hyperbola) = 24.757
Sub−PI = 15.08
Effect of po2
0
5
10
15
8
10
12
14
16
18
po2

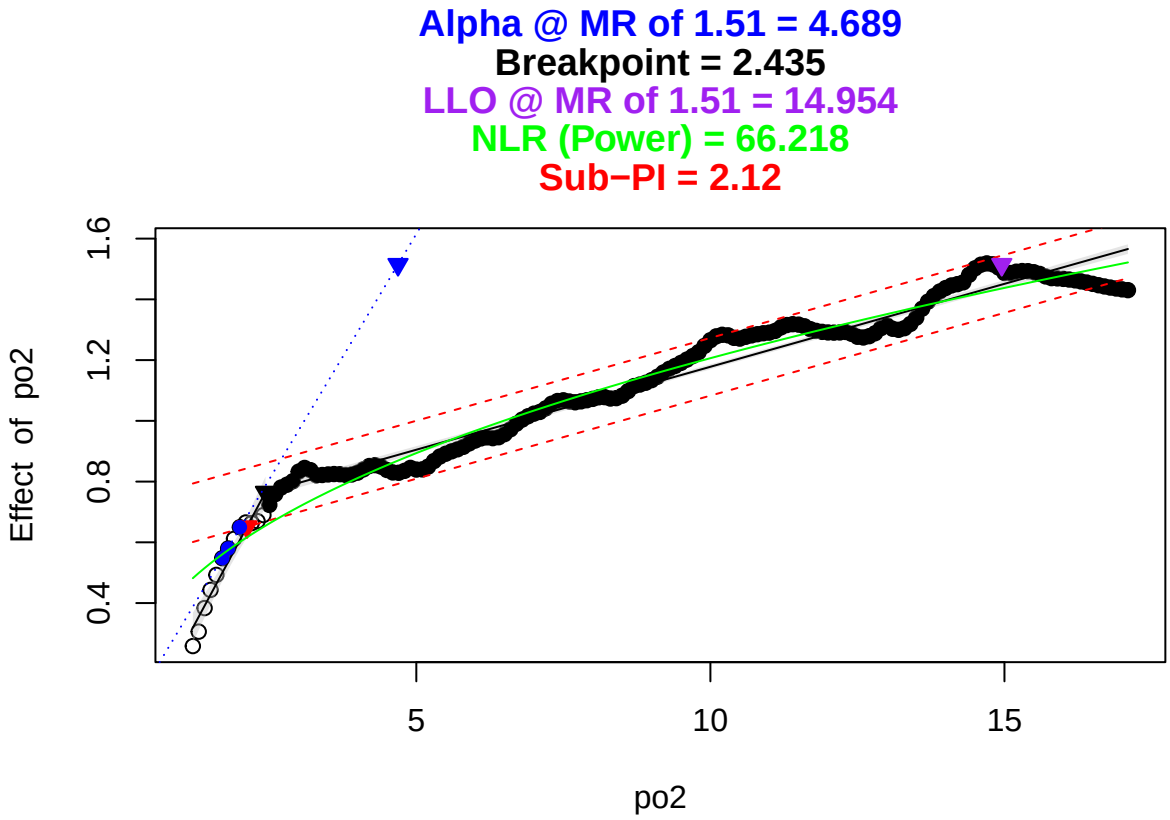

tbocto 1000 pcrit tank 3 and 4 day 7 8−19−21−ch1.txt
mass= 13
resp vol=1
drop time=7.9 hr
O2 drop=194.1
rough mean MO2=1.9
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
25000
pcrit.raw$times

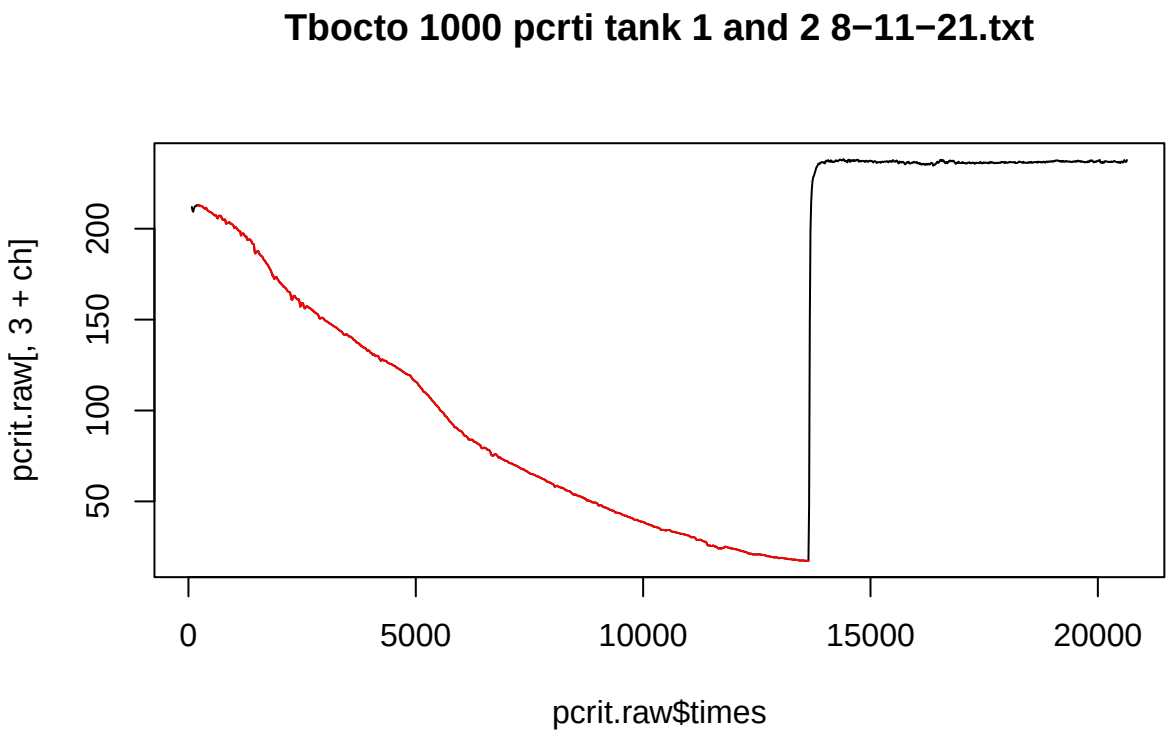

Alpha @ MR of 3.21 = 8.306
Breakpoint = 9
LLO @ MR of 3.21 = 9.657
NLR (Pareto) = 6.088
Sub−PI = 6.81
Effect of po2
0.5
1.5
2.5
3.5
5
10
15
po2

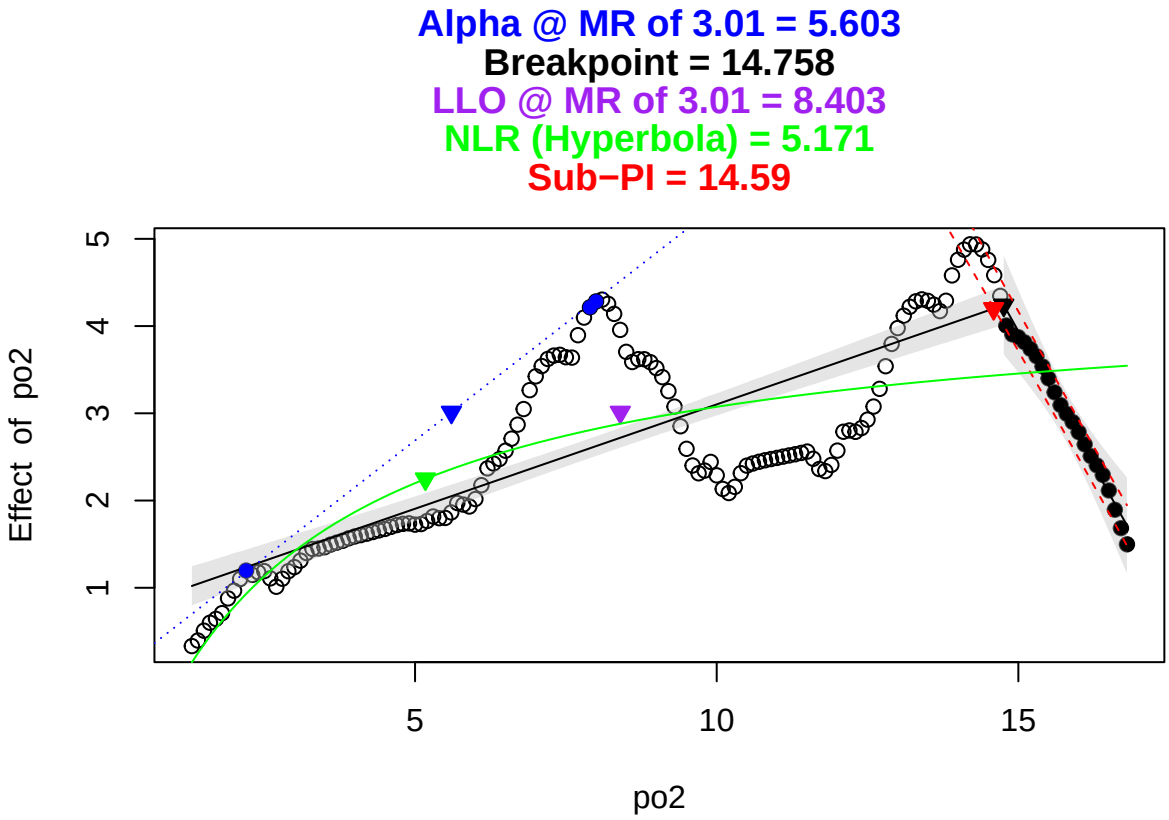

tbocto 1000 pcrit tank 3 and 4 day 7 8−19−21−ch1.txt
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
5000
10000
15000
20000
25000
pcrit.raw$times

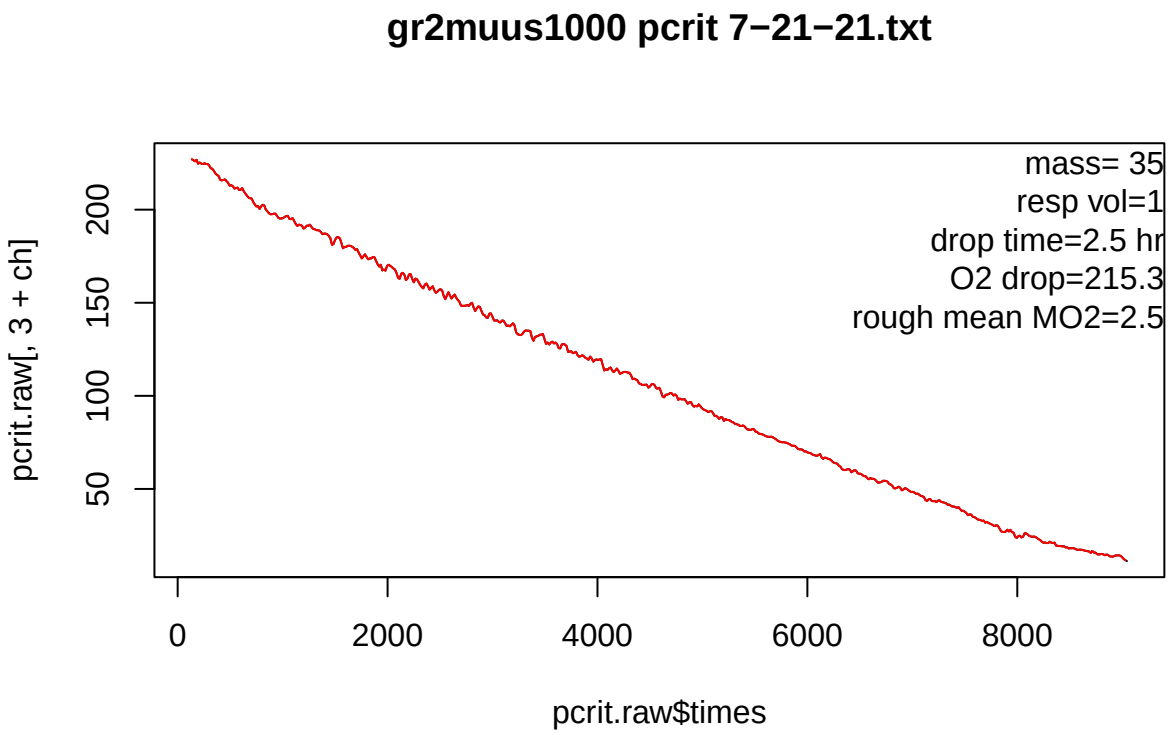

Alpha @ MR of 6.15 = 4.788
Breakpoint = 13.7
LLO @ MR of 6.15 = 7.338
NLR (Hyperbola) = 11.118
Sub−PI = 13.45
Effect of po2
0
5
10
15
5
10
15
po2

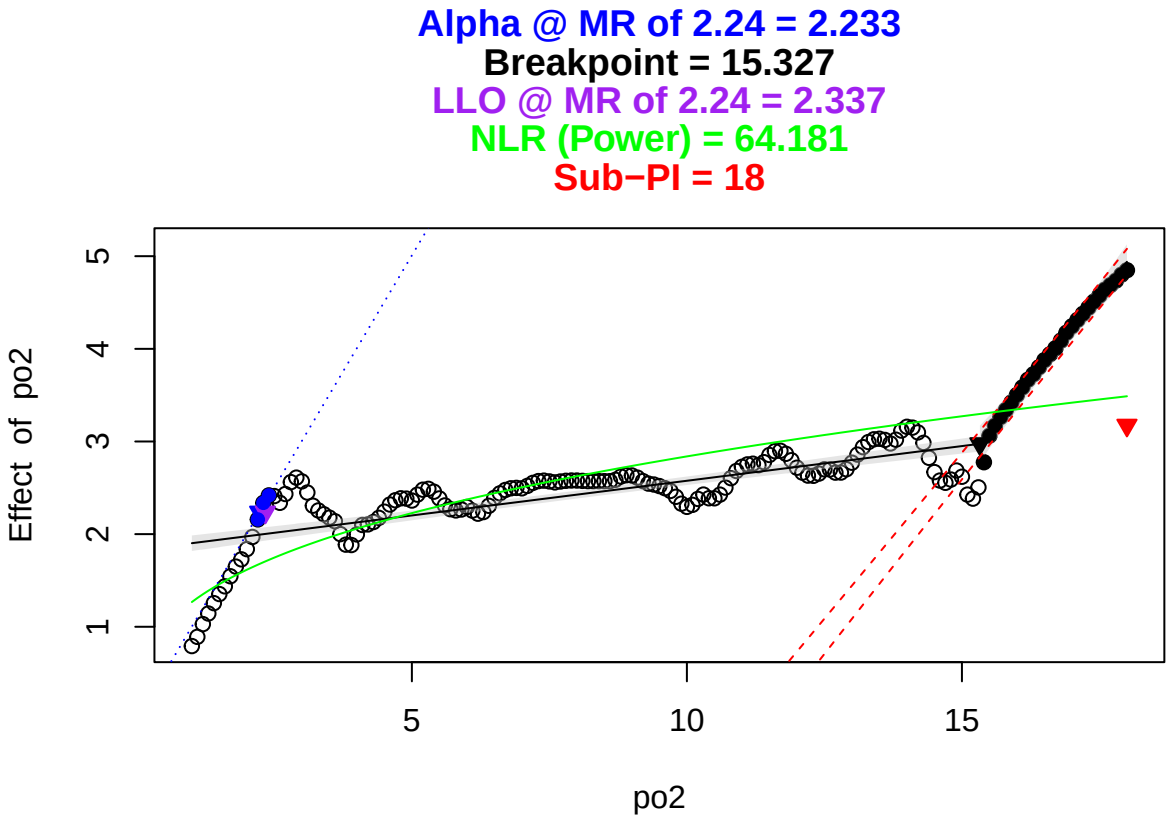

Tbocto 1000 pcrti tank 1 and 2 8−11−21.txt
mass= 36.3
resp vol=1
drop time=5.5 hr
O2 drop=202.2
rough mean MO2=1
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
pcrit.raw$times

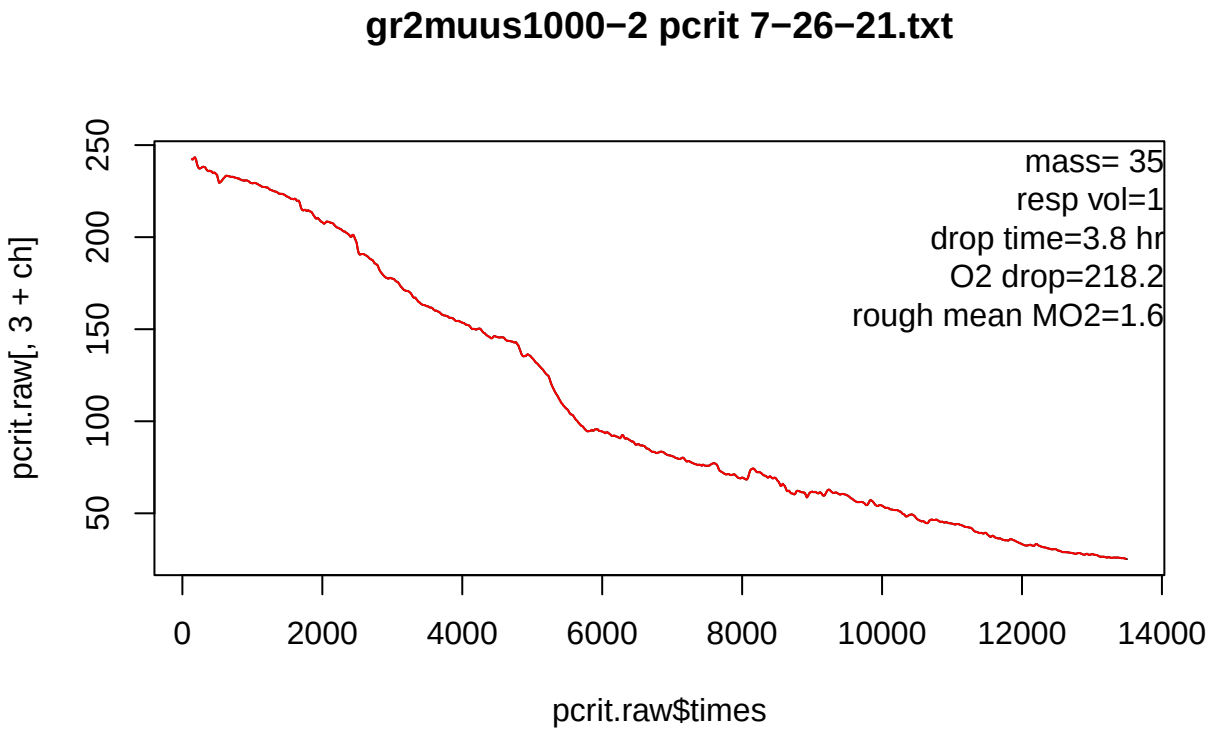

Alpha @ MR of 1.51 = 4.689
Breakpoint = 2.435
LLO @ MR of 1.51 = 14.954
NLR (Power) = 66.218
Sub−PI = 2.12
Effect of po2
0.4
0.8
1.2
1.6
5
10
15
po2

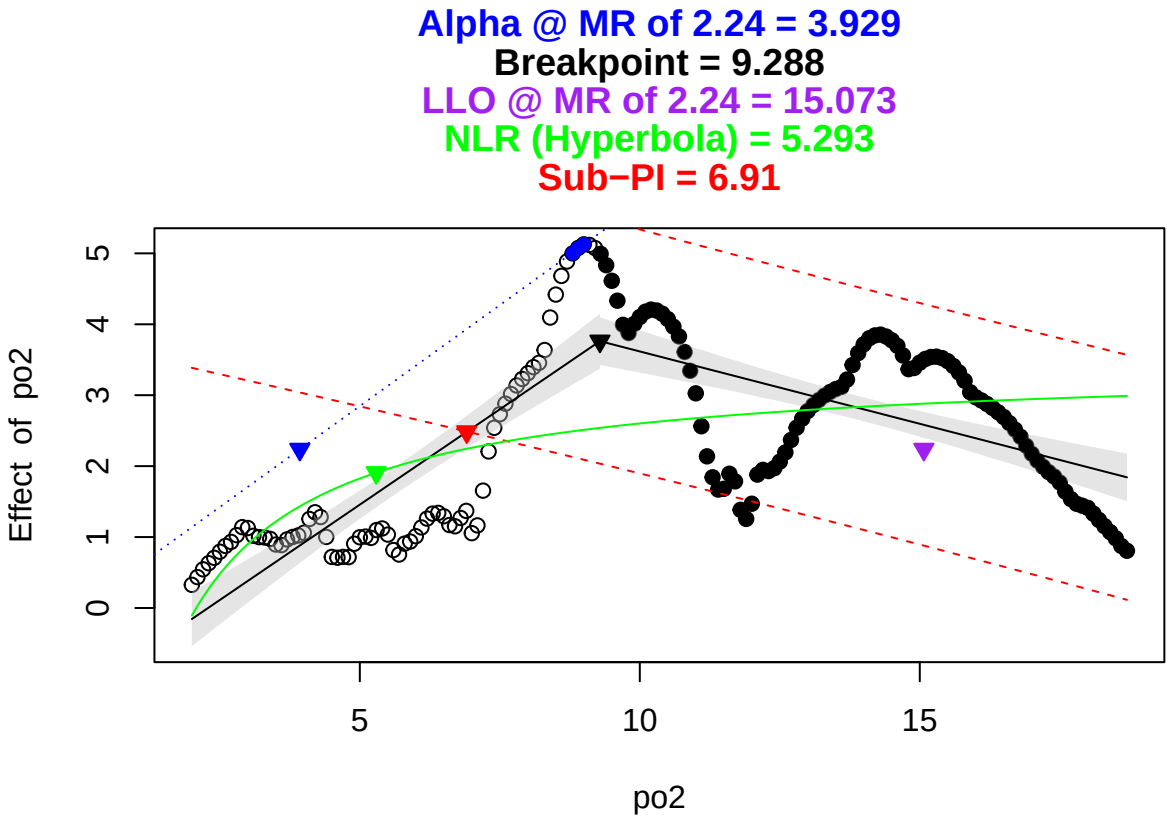

Tbocto 1000 pcrti tank 1 and 2 8−11−21.txt
pcrit.raw[, 3 + ch]
50
100
150
200
0
5000
10000
15000
20000
pcrit.raw$times

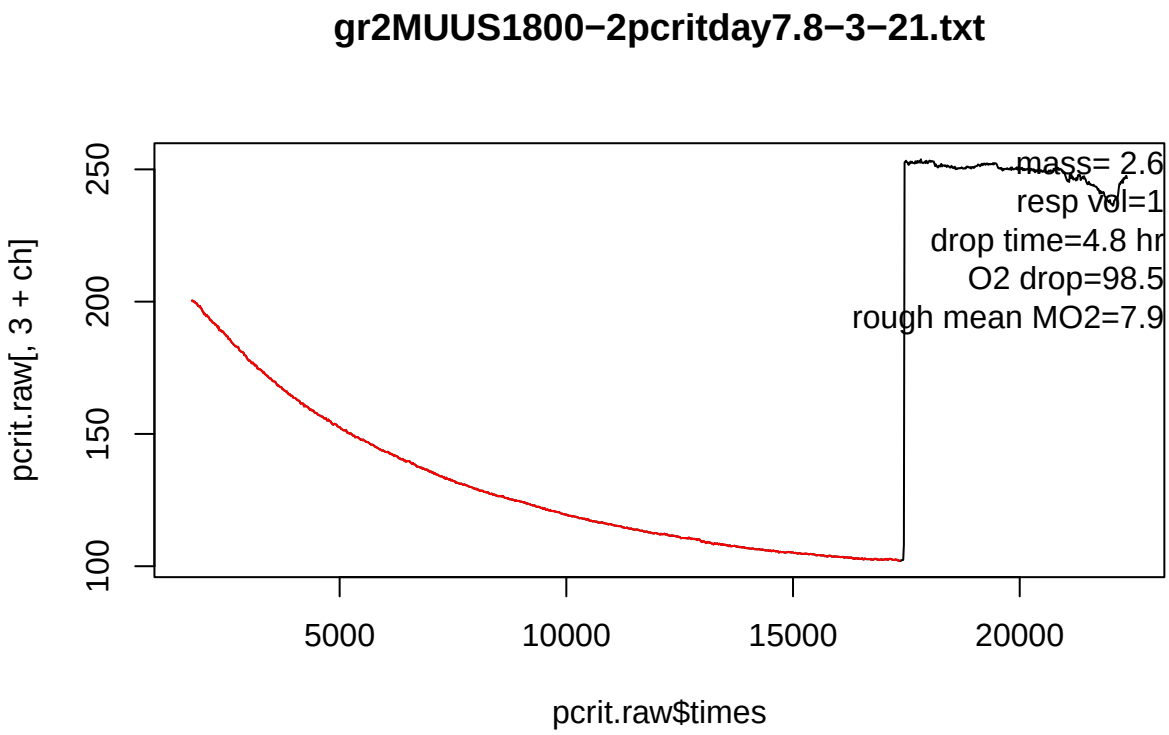

Alpha @ MR of 3.01 = 5.603
Breakpoint = 14.758
LLO @ MR of 3.01 = 8.403
NLR (Hyperbola) = 5.171
Sub−PI = 14.59
Effect of po2
1
2
3
4
5
5
10
15
po2

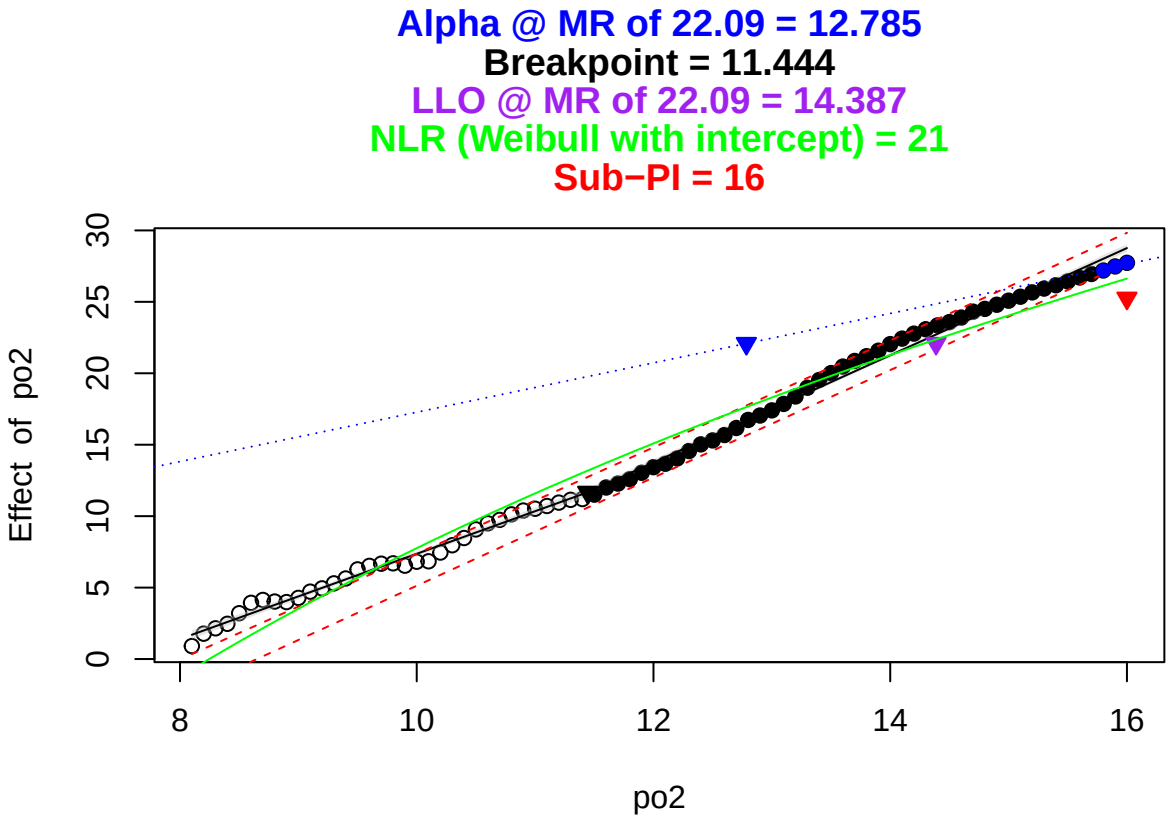

gr2muus1000 pcrit 7−21−21.txt
mass= 35
resp vol=1
drop time=2.5 hr
O2 drop=215.3
rough mean MO2=2.5
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
pcrit.raw$times

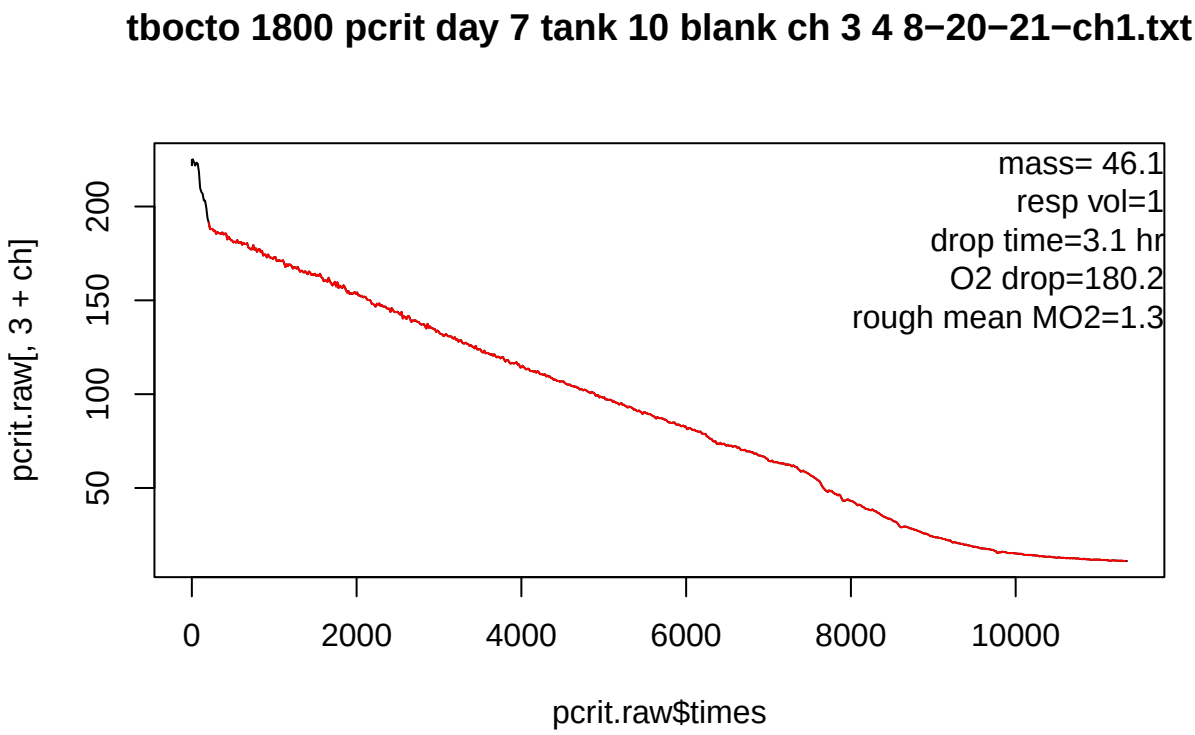

Alpha @ MR of 2.24 = 2.233
Breakpoint = 15.327
LLO @ MR of 2.24 = 2.337
NLR (Power) = 64.181
Sub−PI = 18
Effect of po2
1
2
3
4
5
5
10
15
po2

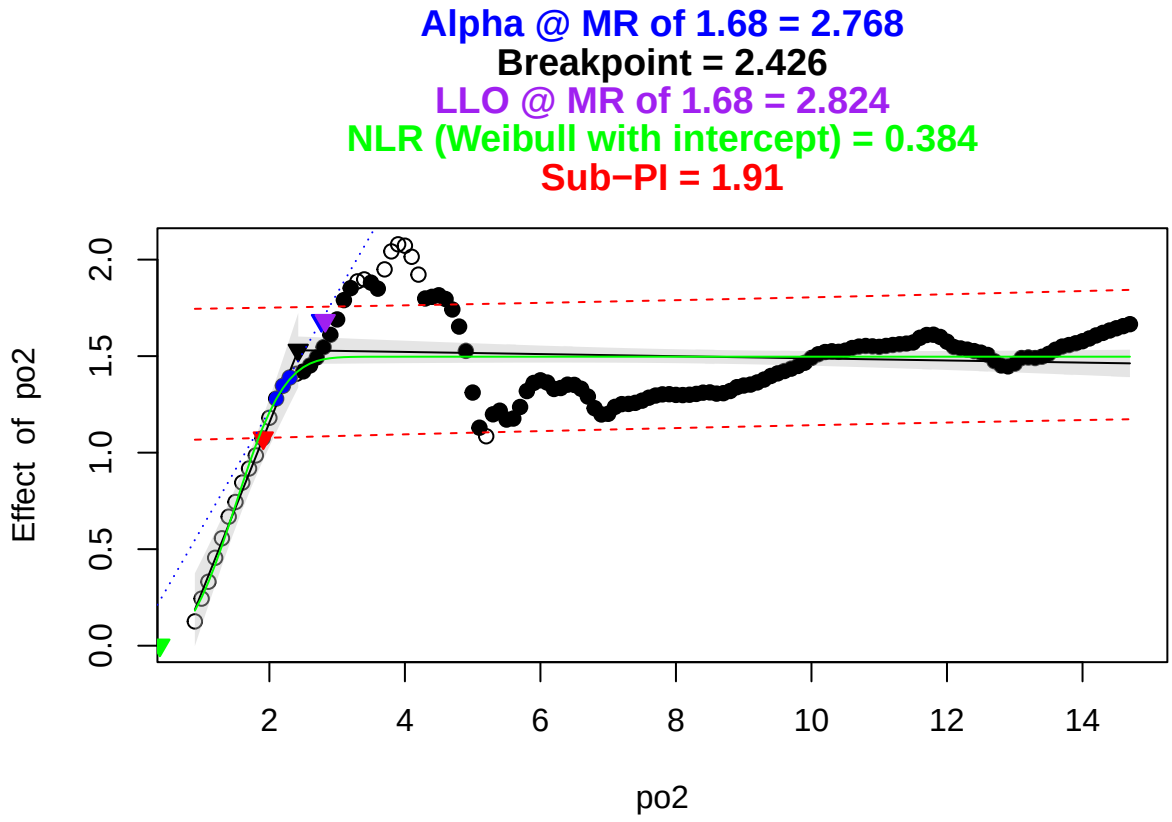

gr2muus1000−2 pcrit 7−26−21.txt
mass= 35
resp vol=1
drop time=3.8 hr
O2 drop=218.2
rough mean MO2=1.6
pcrit.raw[, 3 + ch]
50
100
150
200
250
0
2000
4000
6000
8000
10000
12000
14000
pcrit.raw$times


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
Effect of po2
0
5
10
15
20
25
30
8
10
12
14
16
po2

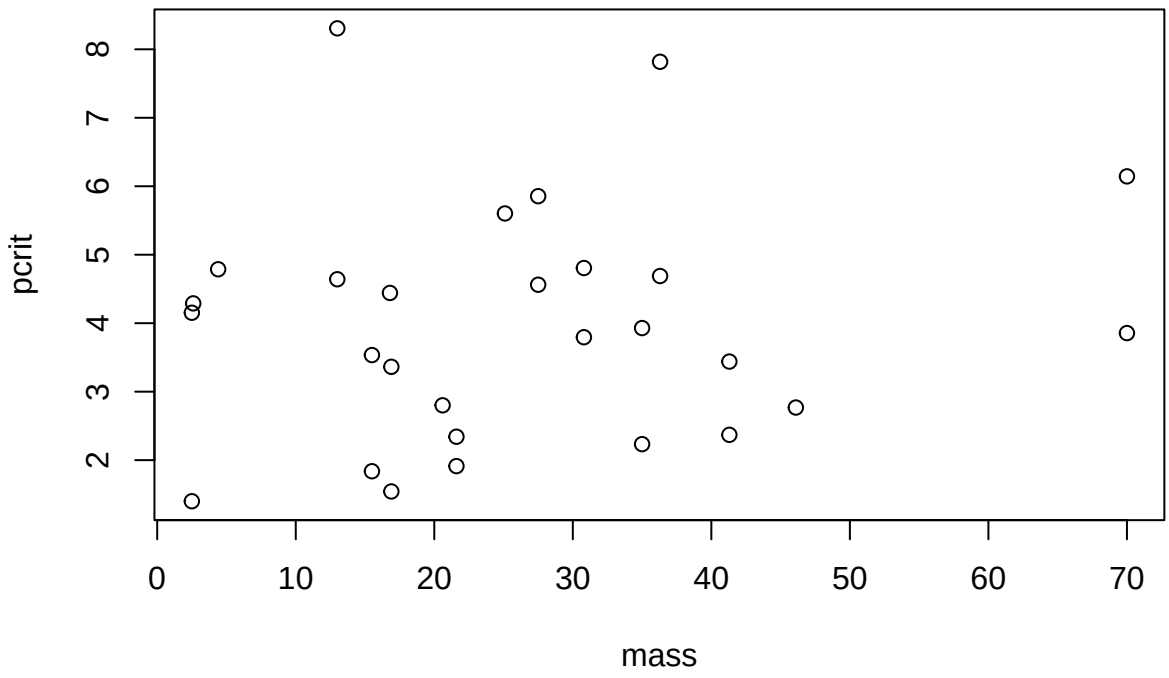

tbocto 1800 pcrit day 7 tank 10 blank ch 3 4 8−20−21−ch1.txt
mass= 46.1
resp vol=1
drop time=3.1 hr
O2 drop=180.2
rough mean MO2=1.3
pcrit.raw[, 3 + ch]
50
100
150
200
0
2000
4000
6000
8000
10000
pcrit.raw$times



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)
```



5 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) 13.6099  1  0.0002250 ***
## mass         0.4546  1  0.5001372
## pco2         0.2625  1  0.6084133
## day         12.0358  1  0.0005219 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

6 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 26.3 3.446430 0.5757775 10 2.163518 4.729343
## 2 1800   1 26.3 3.075144 0.5260269 10 1.903083 4.247205
## 3 1000   7 26.3 4.943982 0.5605456 10 3.695009 6.192956
## 4 1800   7 26.3 4.572696 0.5409960 10 3.367281 5.778110
```

```
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 3.45 2.16 - 4.73
## 3 1000  7 4.94 3.70 - 6.19
## 2 1800  1 3.08 1.90 - 4.25
## 4 1800  7 4.57 3.37 - 5.78
```

```
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	3.45	2.16 - 4.73
1000	7	4.94	3.70 - 6.19
1800	1	3.08	1.90 - 4.25
1800	7	4.57	3.37 - 5.78

7 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
```

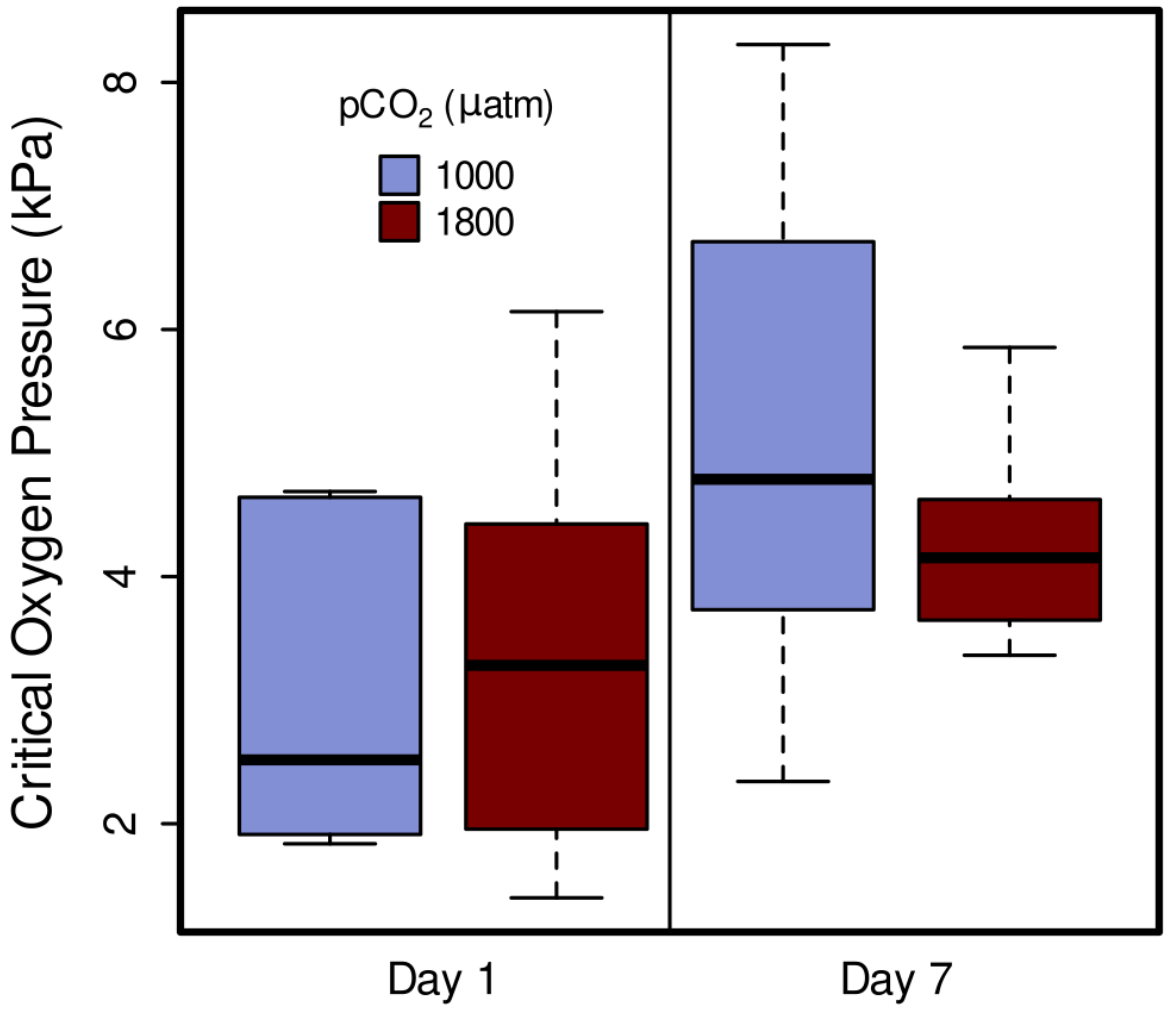


Figure 1: Critical oxygen pressure of Muusoctopus leioderma at differing pCO2 treatments