

Critical Oxygen Pressure (P_{CRIT}) Analysis

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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 MO_2 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 mmHg O_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")

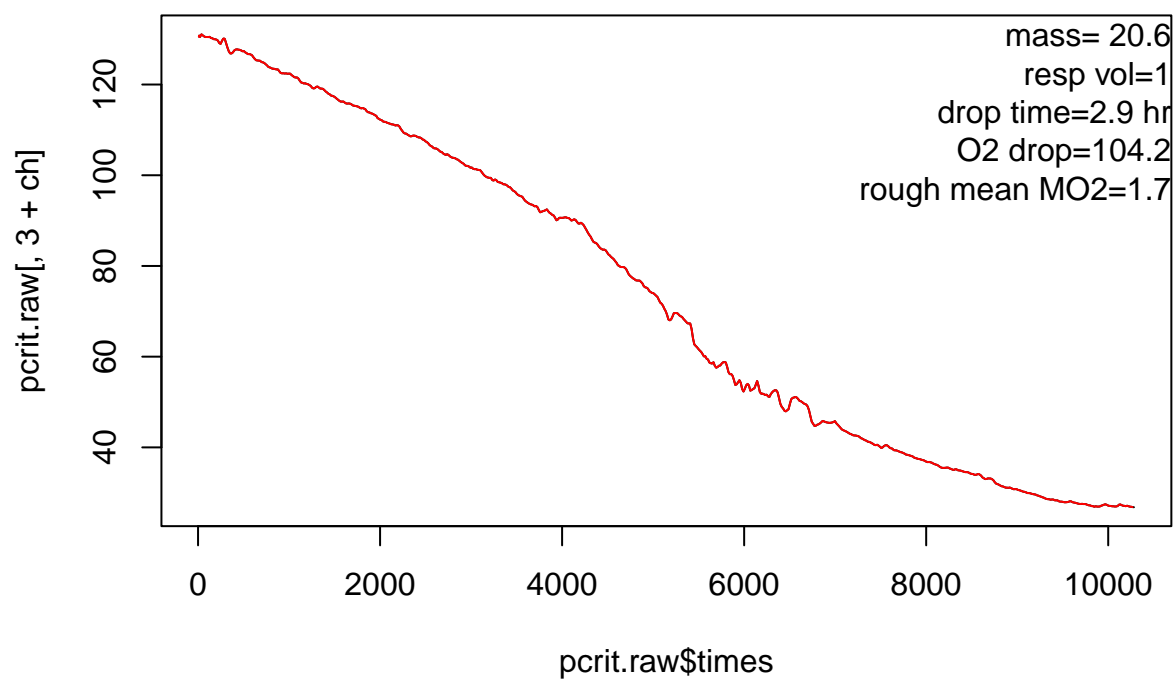
pcrit.resp=resp.closed(pcrit.working,volume=pcrit.log$vol[guess],
  weight=mass,smooth="loess",channel=ch,smooth.span = 0.2)
pcrit.bin=aggregate(pcrit.resp$resp~round(pcrit.resp$po2,1),FUN="mean")
colnames(pcrit.bin)=c("po2","resp")

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)
}

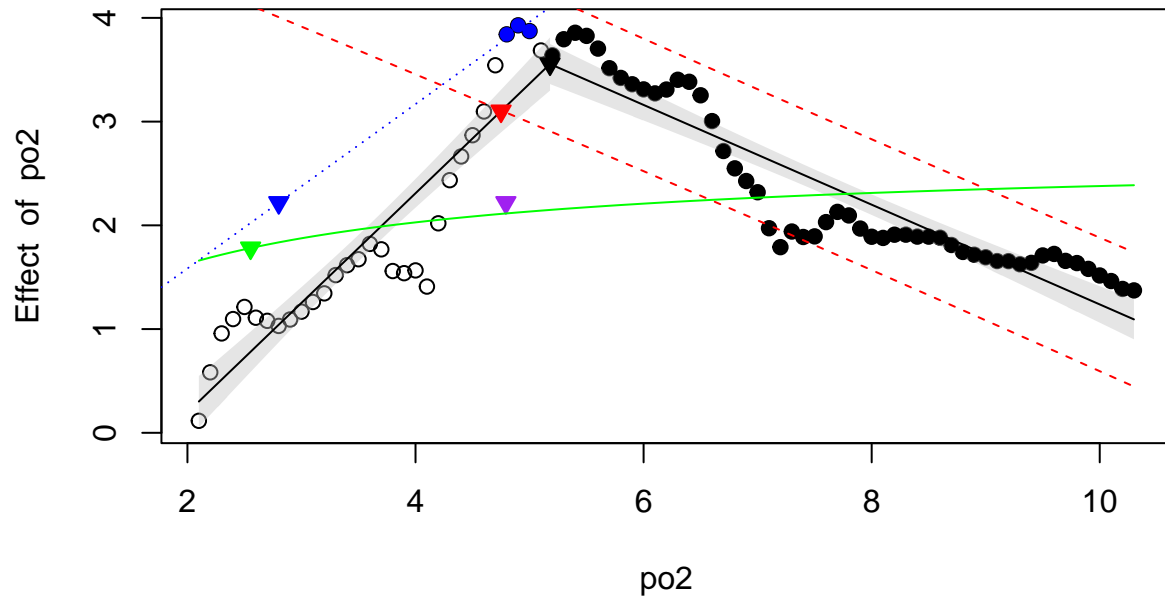
pcrits[co,1]=filename
pcrits[co,2]=pcrit.log$filename[guess]
pcrits[co,3]=octo
pcrits[co,4]=mass
pcrits[co,5]=pcrits$pco2[guess]
pcrits[co,6]=pcrits$day[guess]
pcrits[co,7]=rmr
if (min(pcrit.working[,3+ch])<50){
  if(max(pcrit.bin$resp)>rmr){
    pcrits[co,8]=as.numeric(calc_pcrit(pcrit.bin$po2,pcrit.bin$resp,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

```

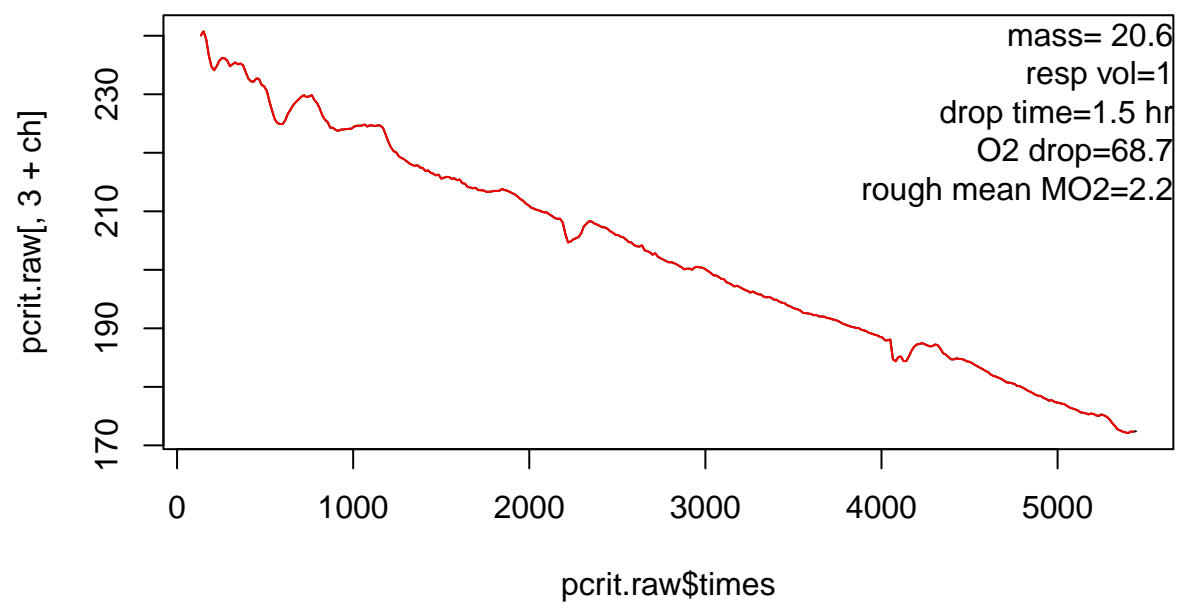
Gr1 Muus 1000-2 pcrit 7-27-21 B.txt



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



Gr1 Muus 1000-2 pcrit 7-27-21.txt



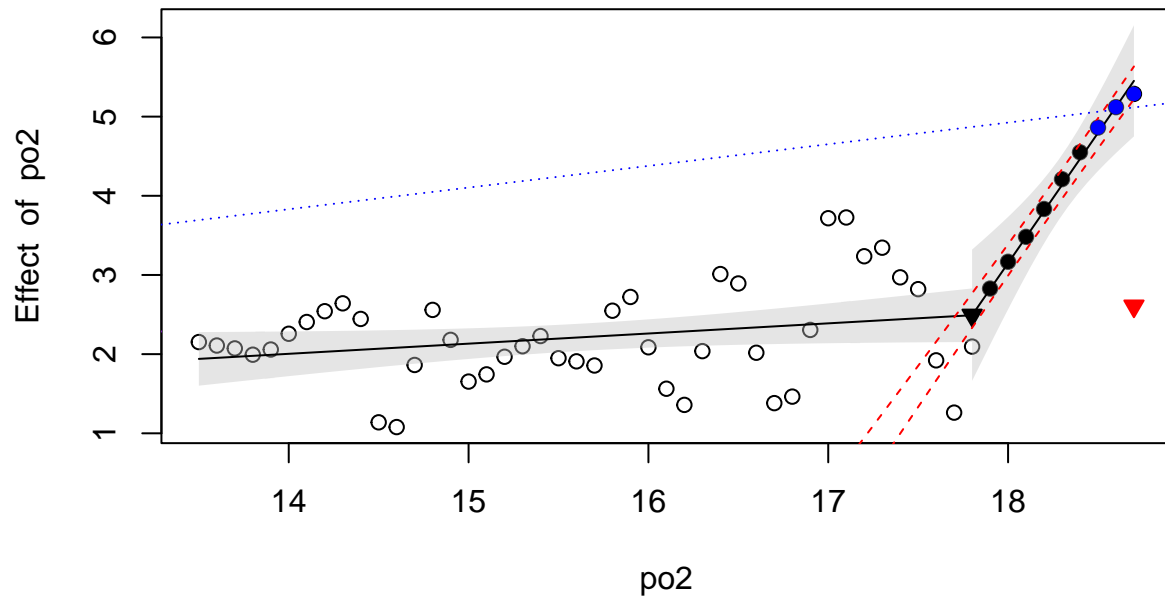
Alpha @ MR of 2.22 = 8.109

Breakpoint = 17.8

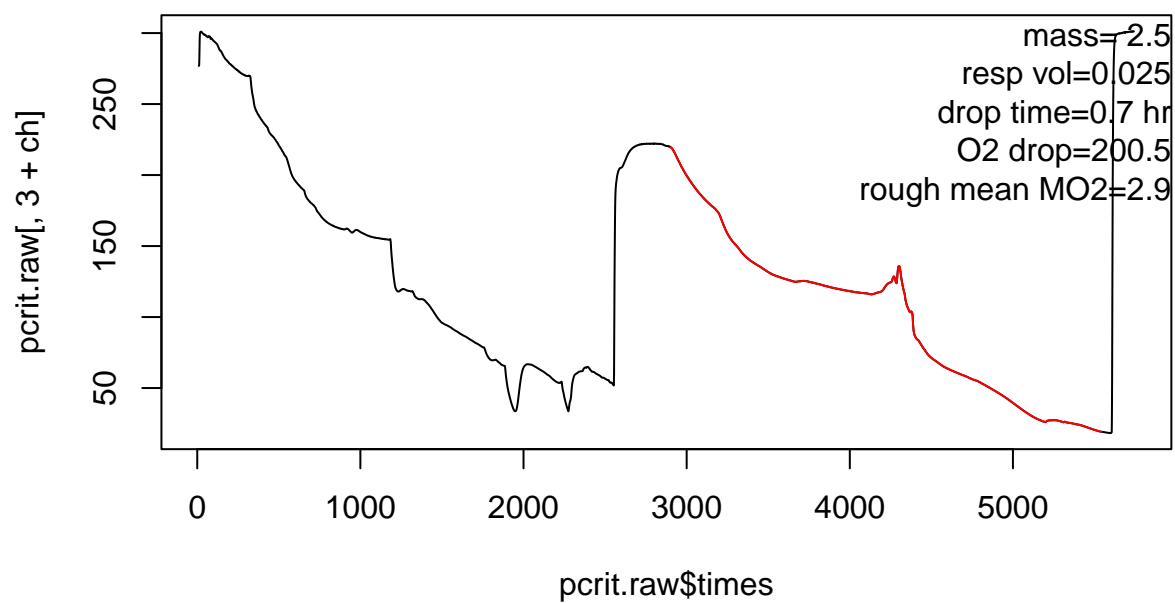
LLO @ MR of 2.22 = 13.235

NLR () = NA

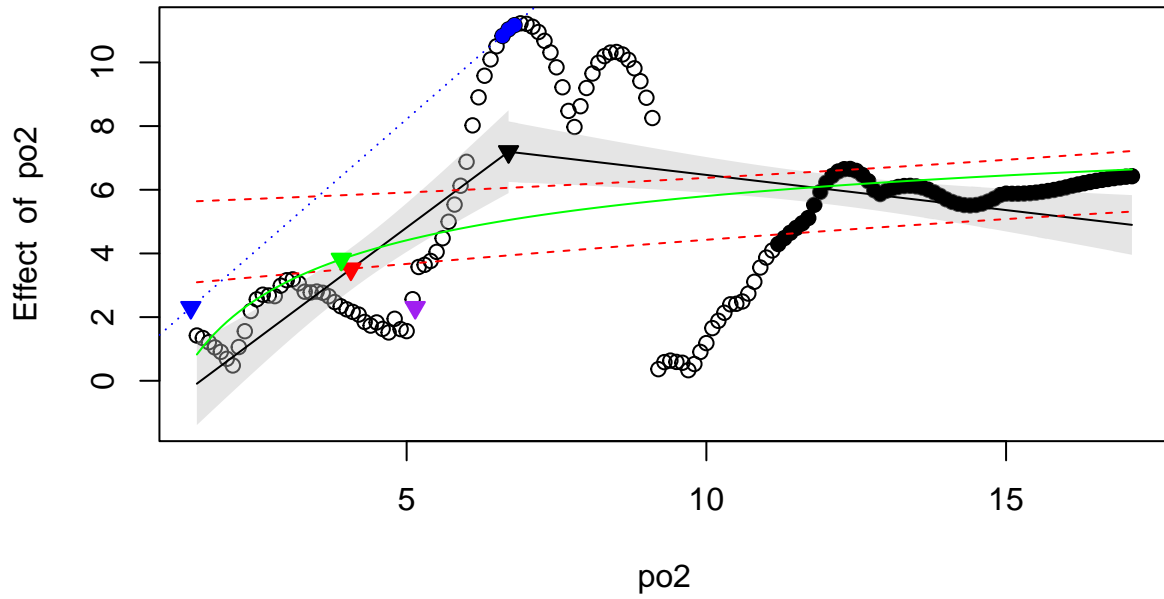
Sub-PI = 18.7



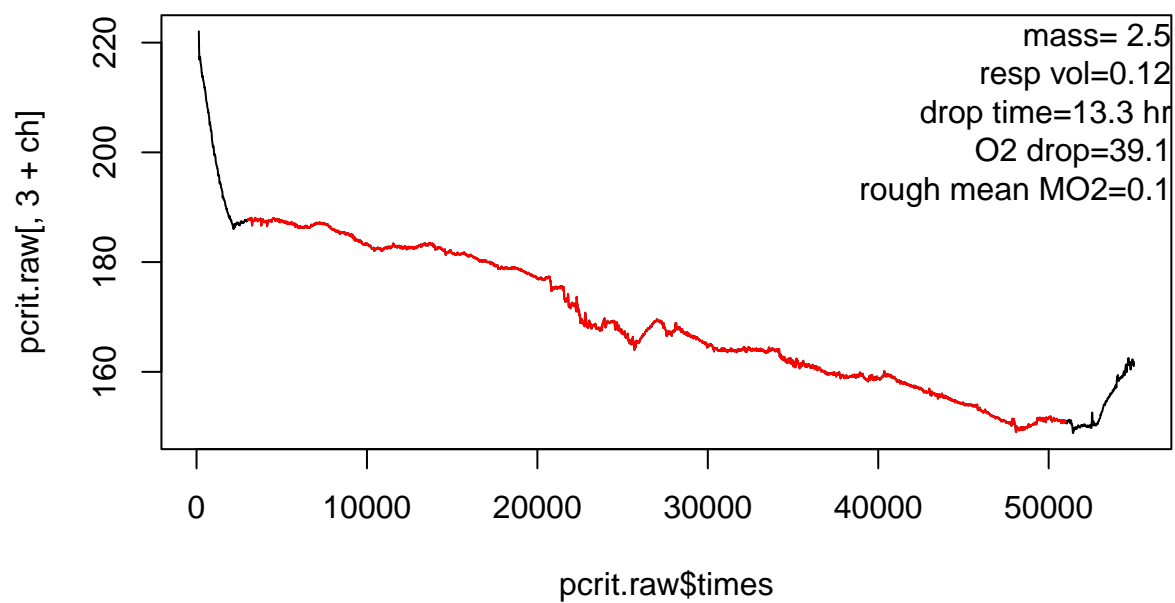
Gr1 Muus 1800-2 pcrit 25 ml jar 7-29-21 ch2 blank.txt



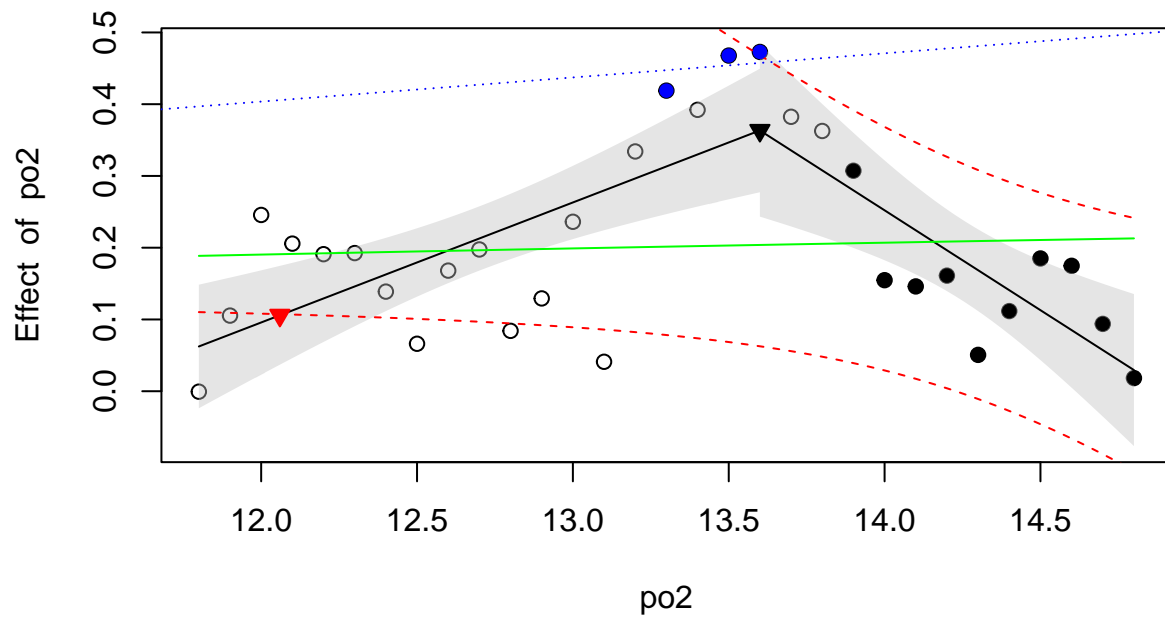
Alpha @ MR of 2.3 = 1.4
Breakpoint = 6.7
LLO @ MR of 2.3 = 5.143
NLR (Pareto) = 3.91
Sub-PI = 4.07



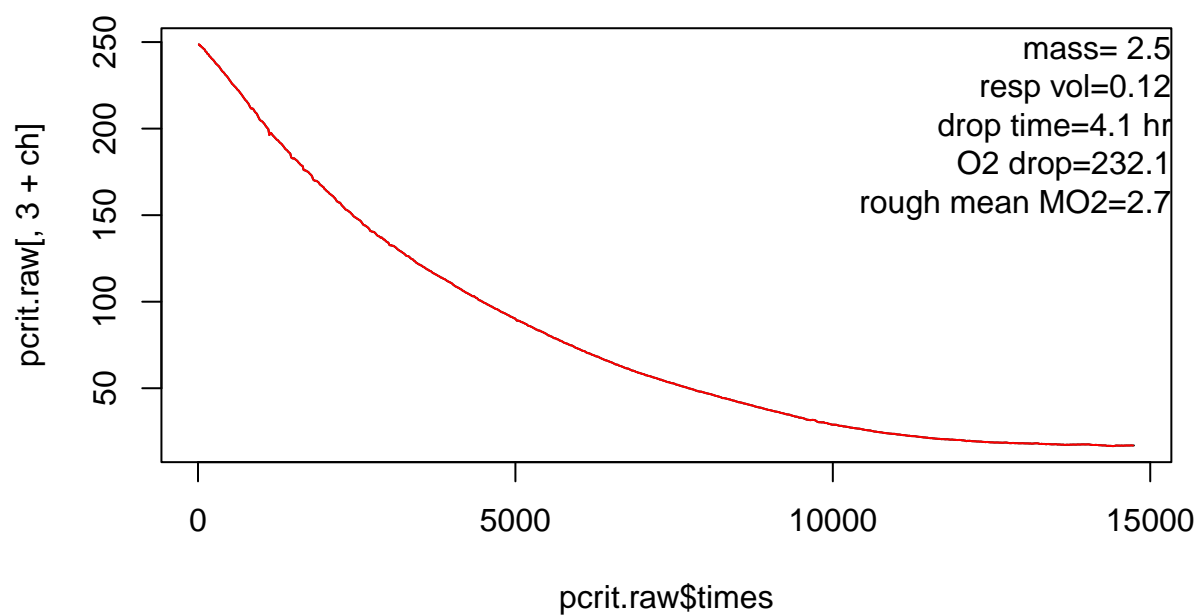
Gr1 Muus 1800-2 pcrit 7-28-21.txt



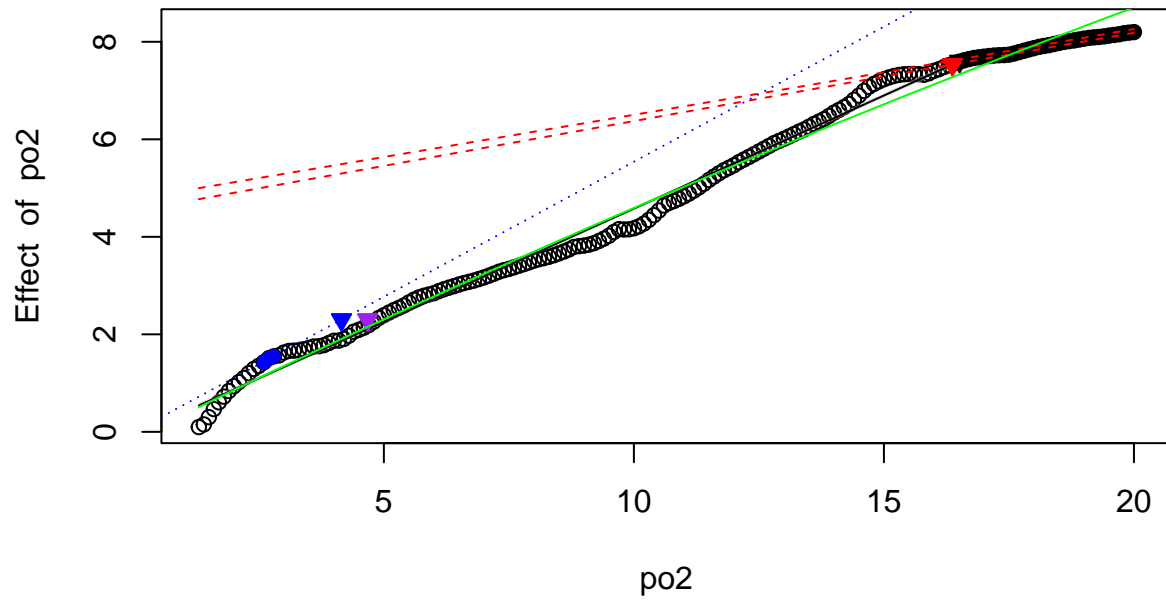
Alpha @ MR of 0.14 = 4.172
Breakpoint = 13.6
LLO @ MR of NA = NA
NLR (Michaelis-Menten) = 0.829
Sub-PI = 12.06



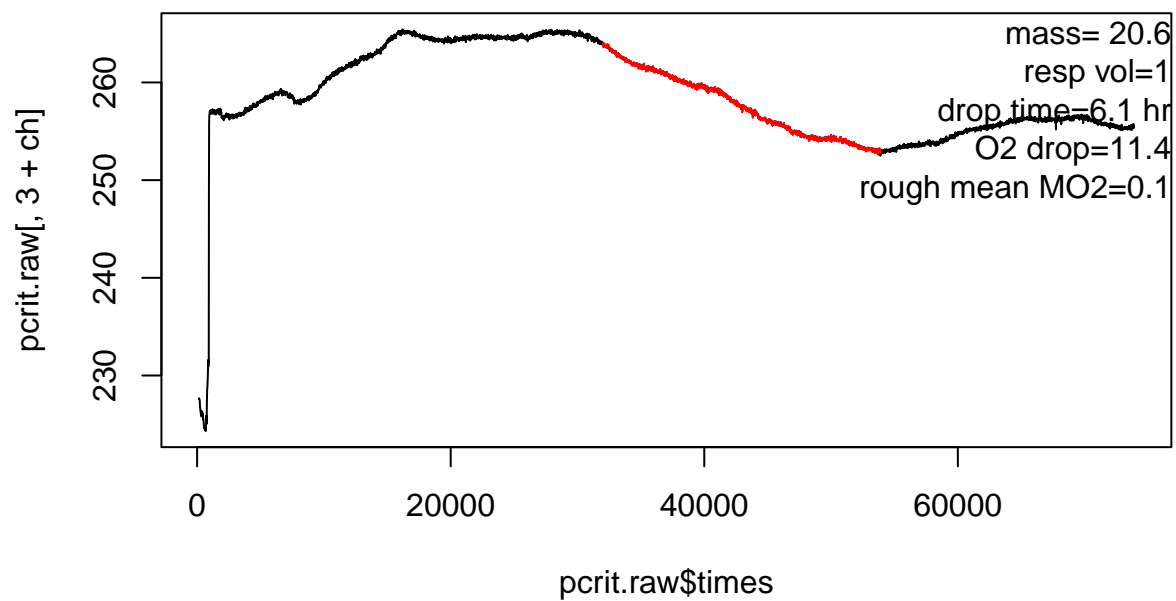
GR1 Muus 1800-2 pcrit day7 8-3-21.txt



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



GR1 Muus1000 7day-7-26-21.txt



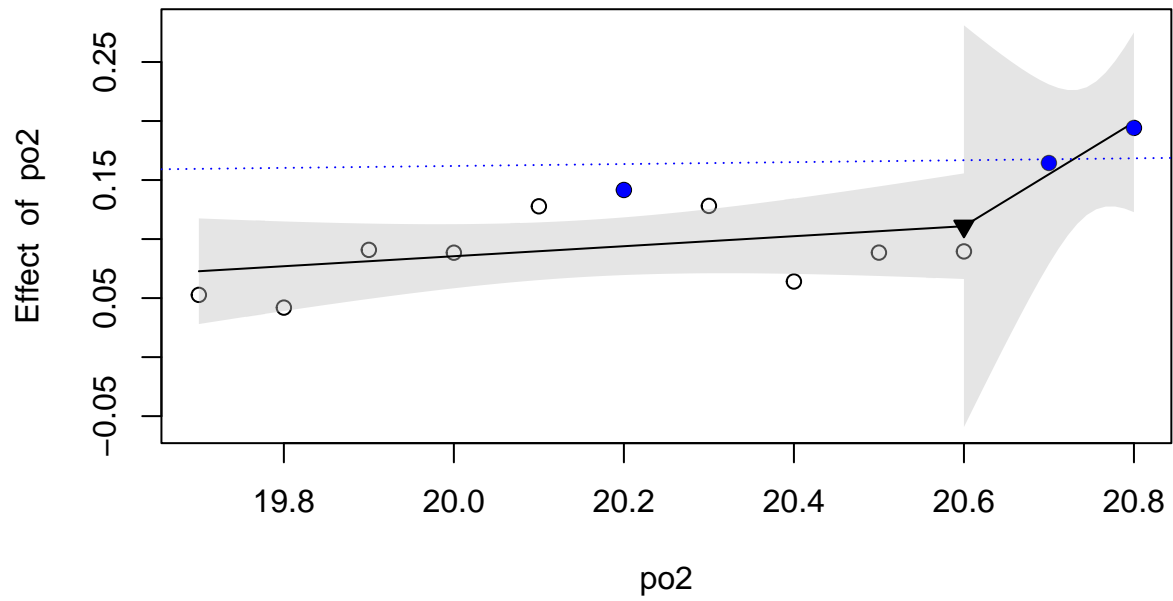
Alpha @ MR of 0.18 = 22.147

Breakpoint = 20.6

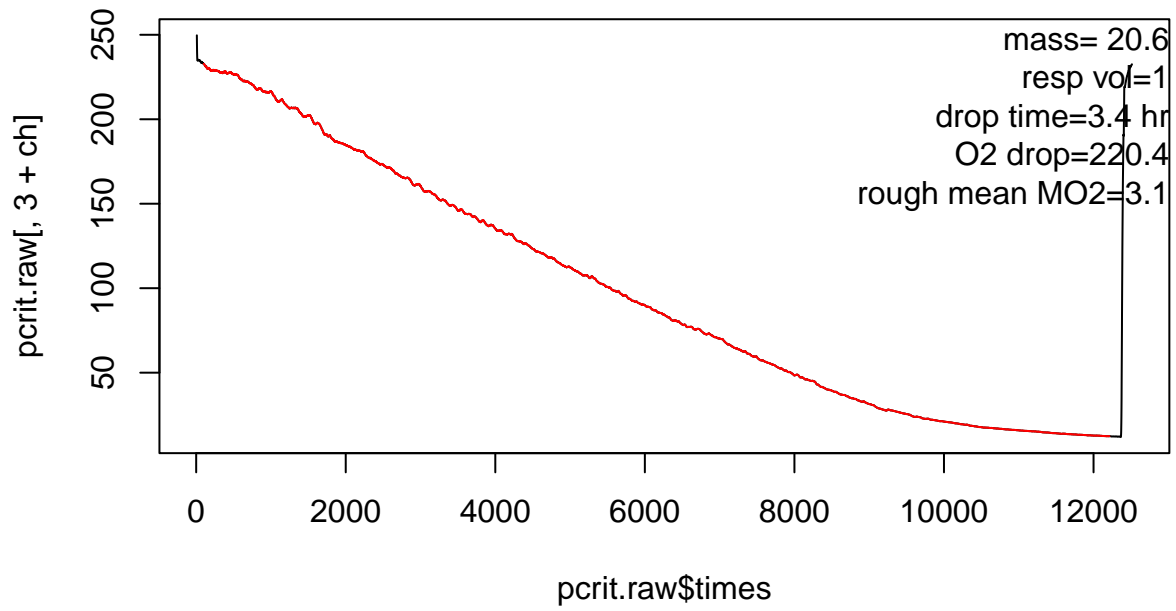
LLO @ MR of NA = NA

NLR () = NA

Sub-PI =



GR1 Muus1000 pcrit 7-21-21.txt



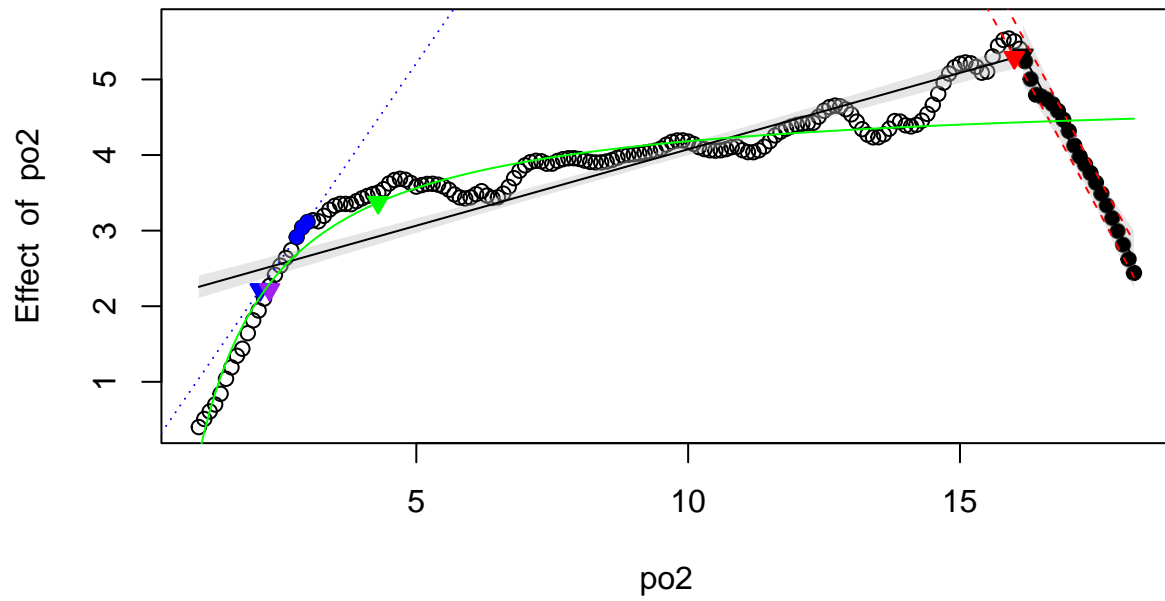
Alpha @ MR of 2.22 = 2.128

Breakpoint = 16.161

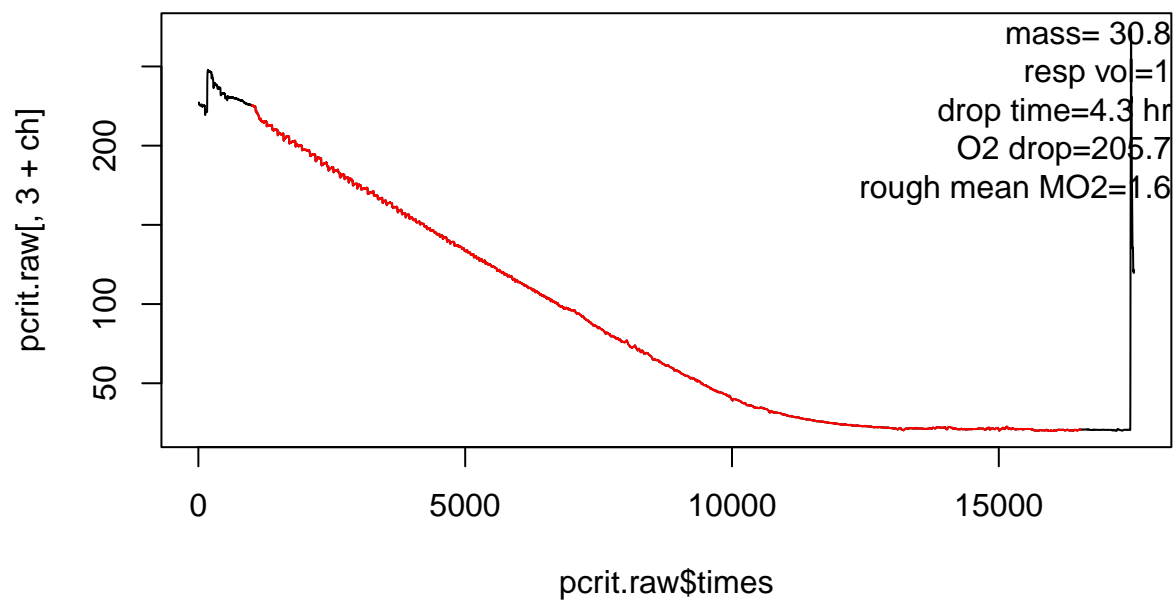
LLO @ MR of 2.22 = 2.3

NLR (Hyperbola) = 4.298

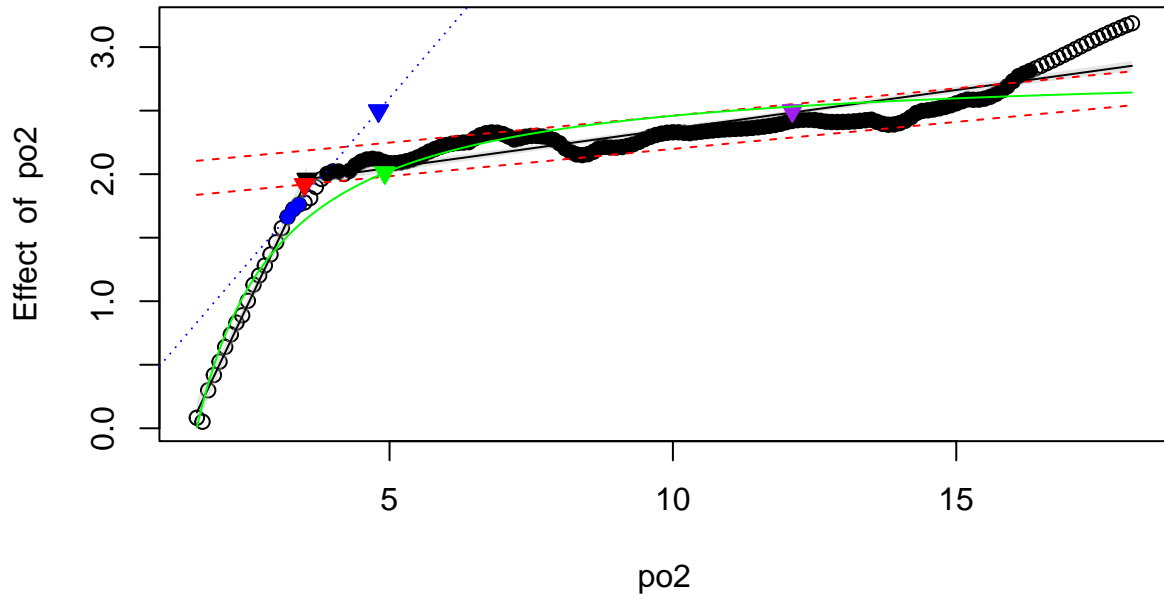
Sub-PI = 16



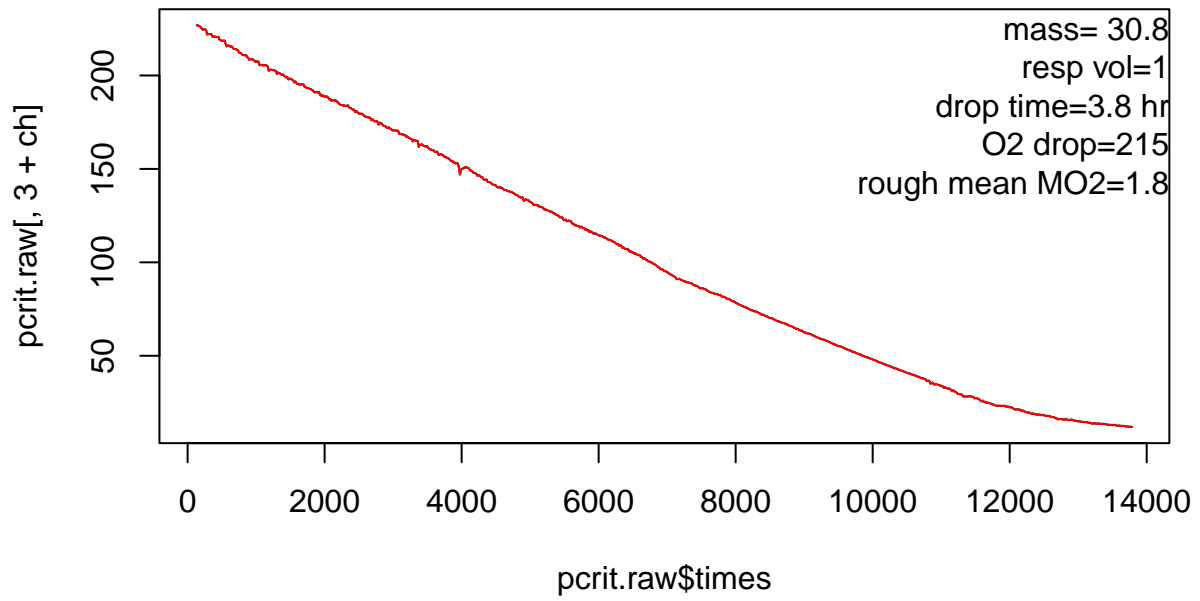
GR1 Muus1800 7day-pcrit 7-20-21.txt



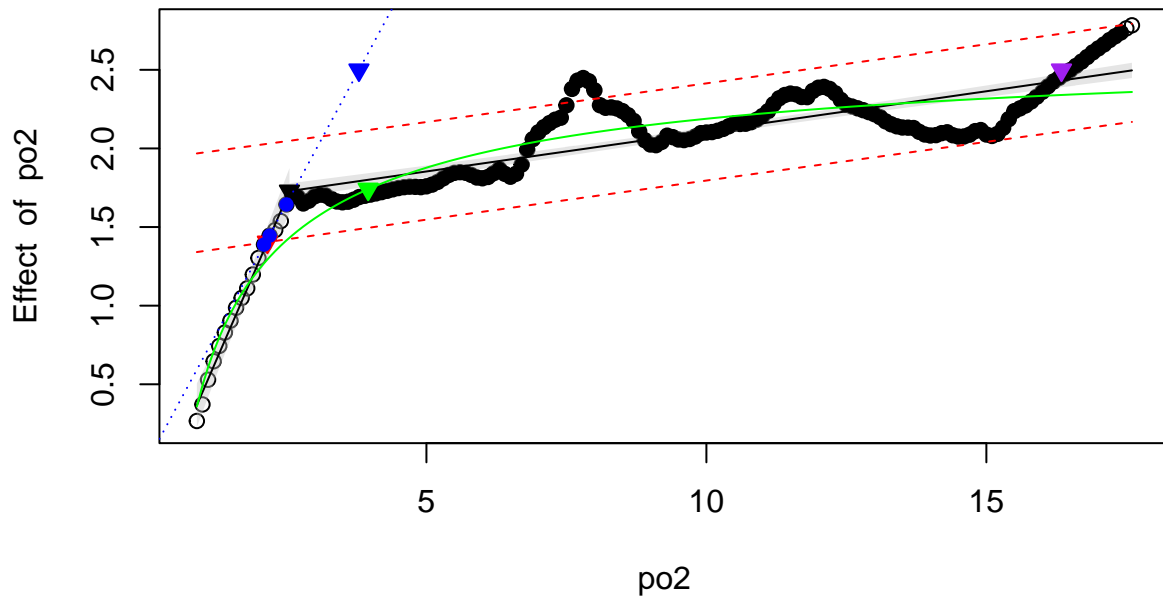
Alpha @ MR of 2.5 = 4.806
Breakpoint = 3.545
LLO @ MR of 2.5 = 12.106
NLR (Pareto) = 4.917
Sub-PI = 3.5



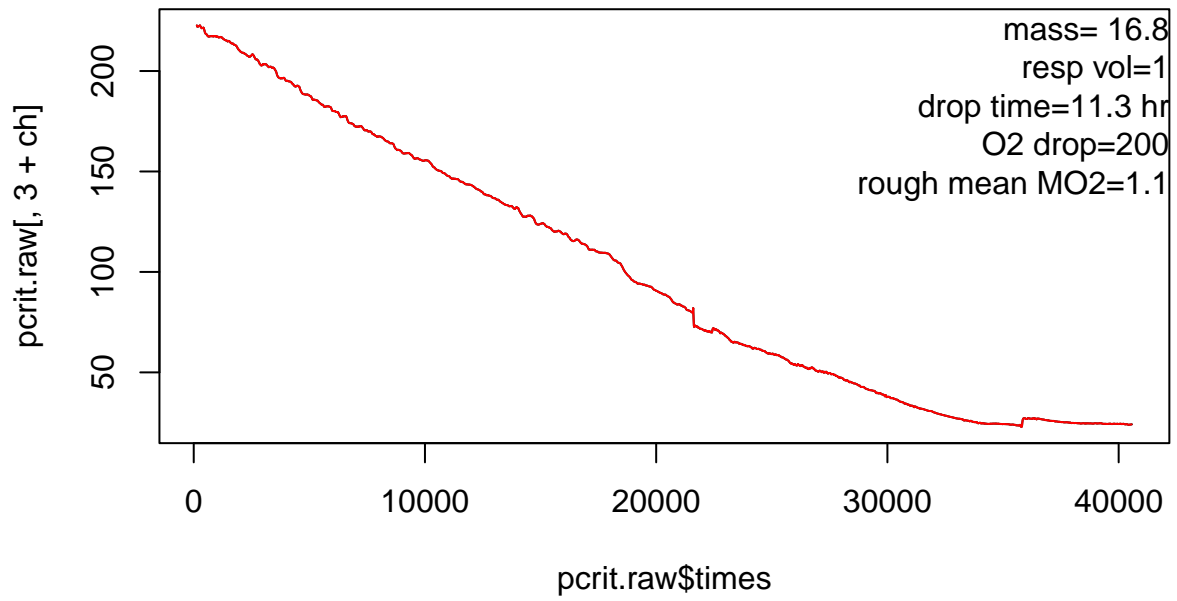
GR1 Muus1800 pcrit 7-13-21.txt



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



gr2muus1800 7day pcrit 7-20-21.txt



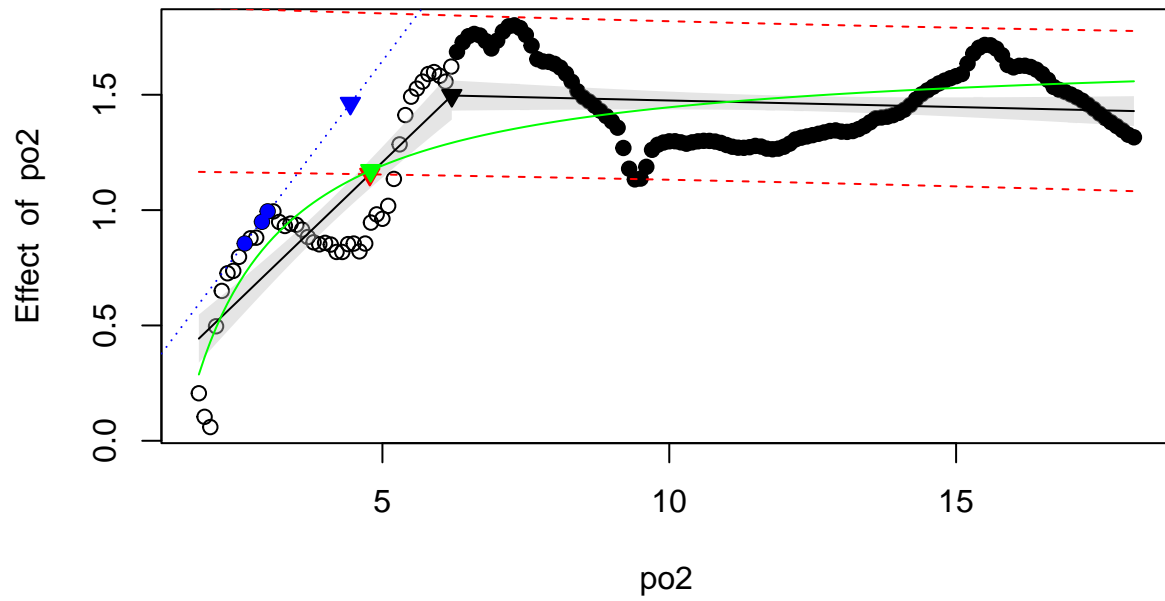
Alpha @ MR of 1.46 = 4.442

Breakpoint = 6.209

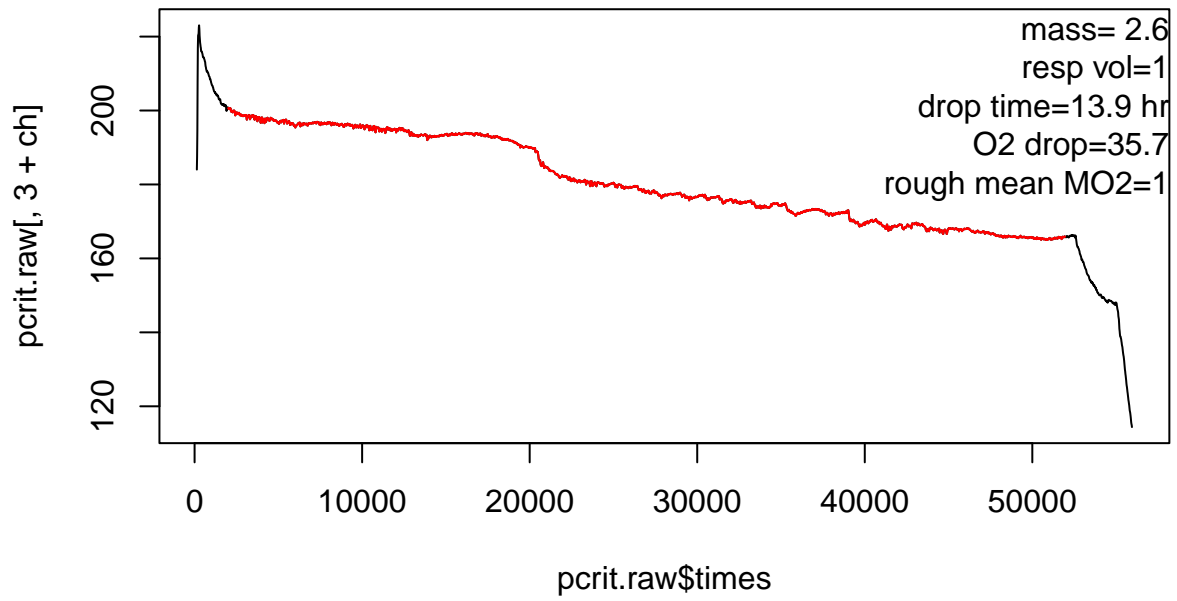
LLO @ MR of NA = NA

NLR (Pareto) = 4.796

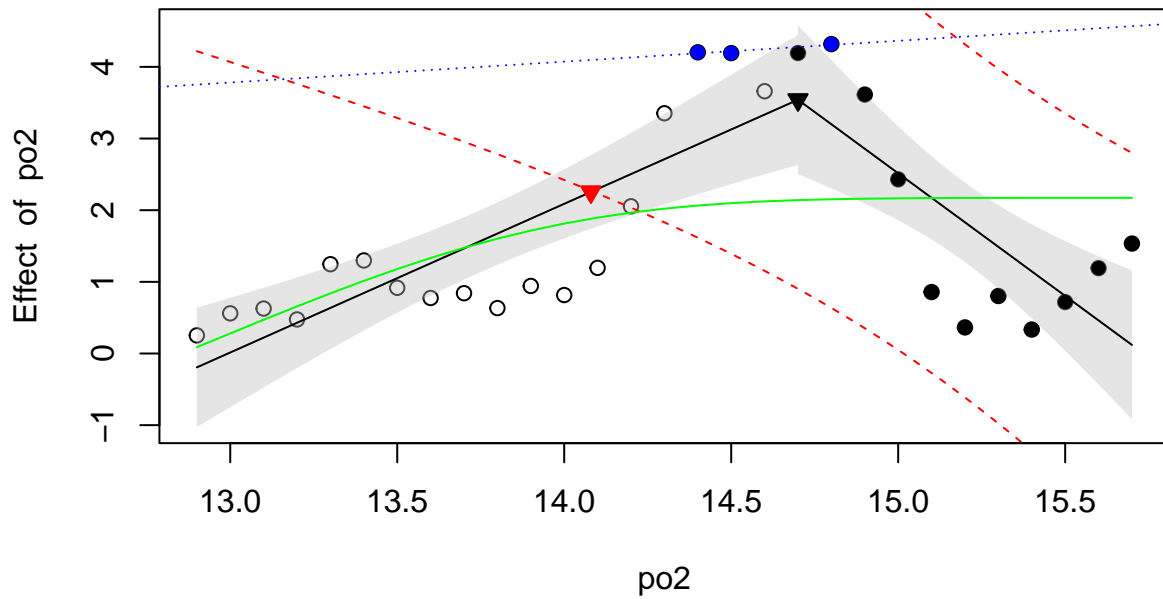
Sub-PI = 4.78



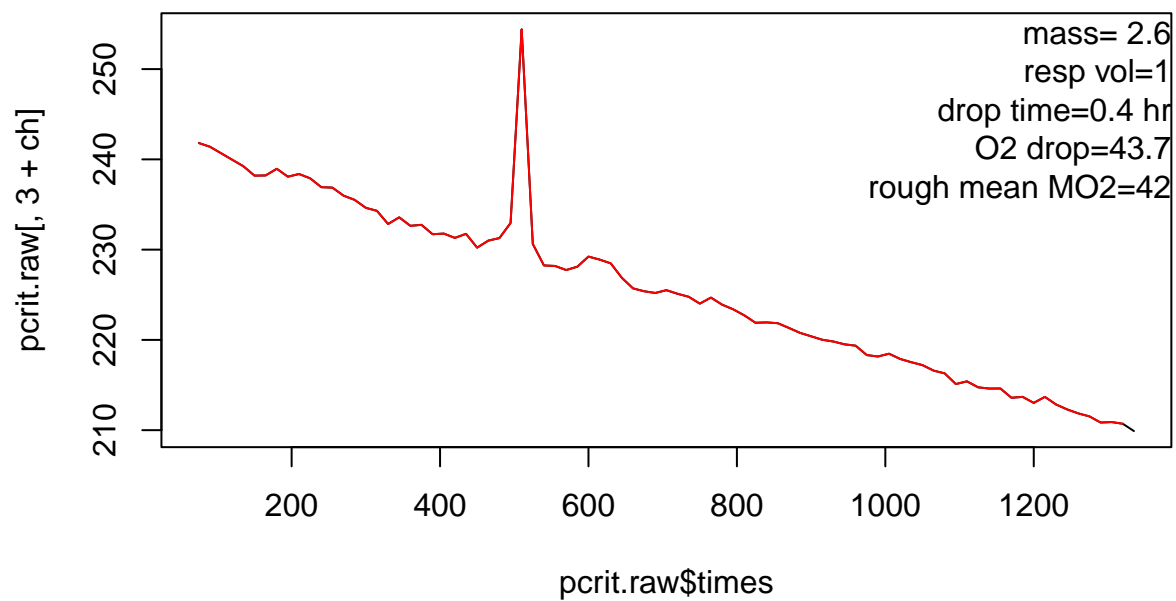
gr2muus1800-2 pcrit 7-28-21.txt



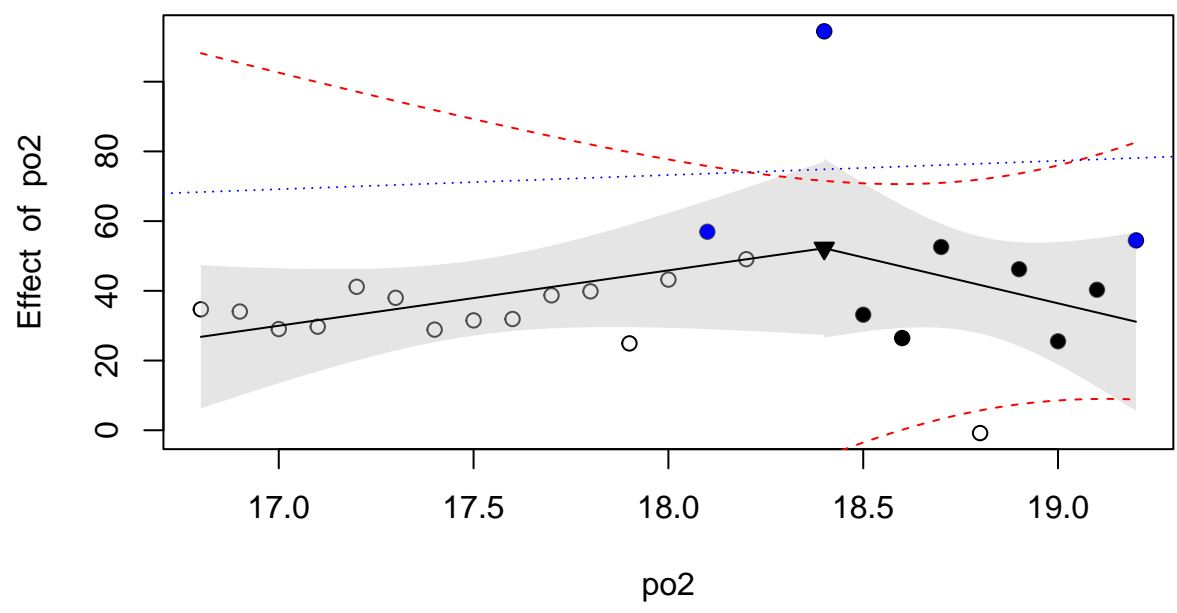
Alpha @ MR of 1.85 = 6.361
 Breakpoint = 14.7
 LLO @ MR of NA = NA
 NLR (Weibull with intercept) = 10.283
 Sub-PI = 14.08



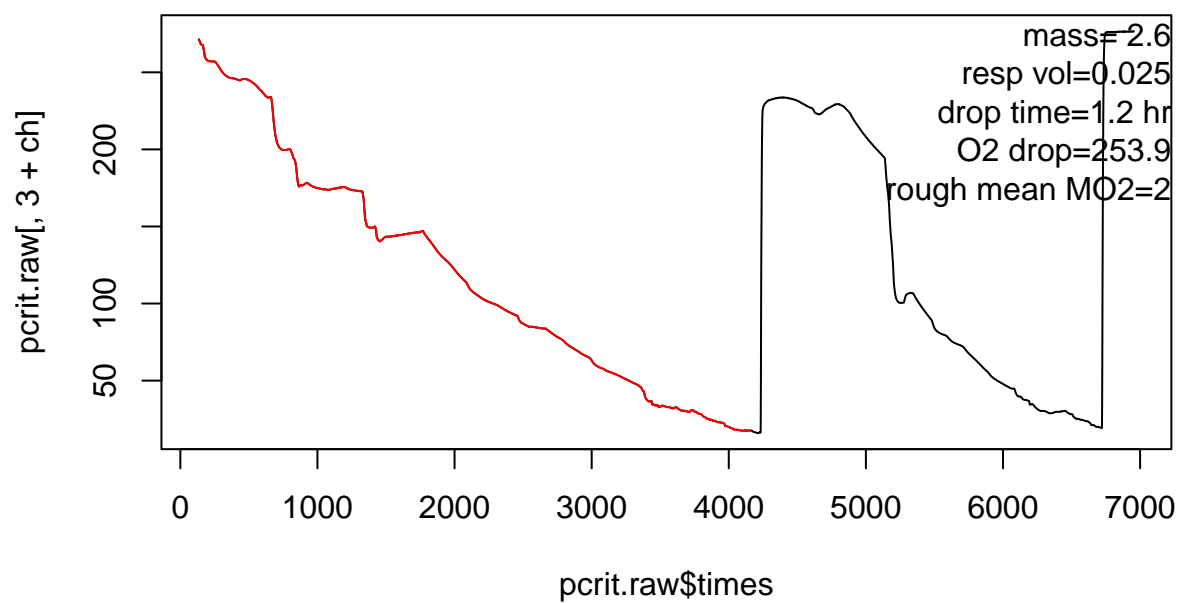
gr2muus1800-2 pcrit day7 8-3-21.txt



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



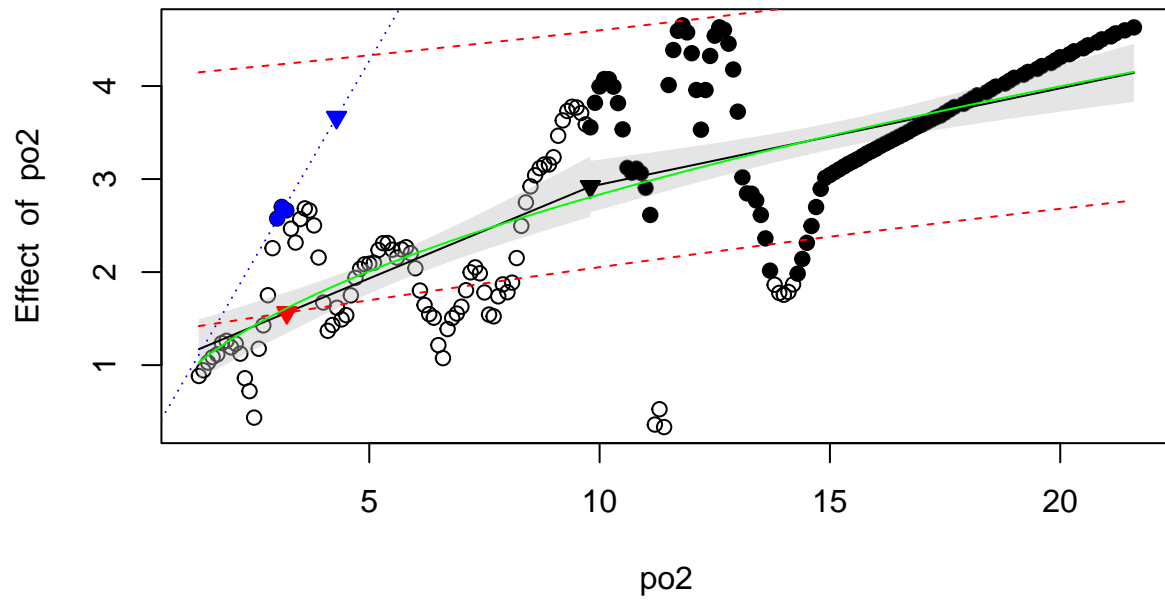
Alpha @ MR of 3.66 = 4.289

Breakpoint = 9.8

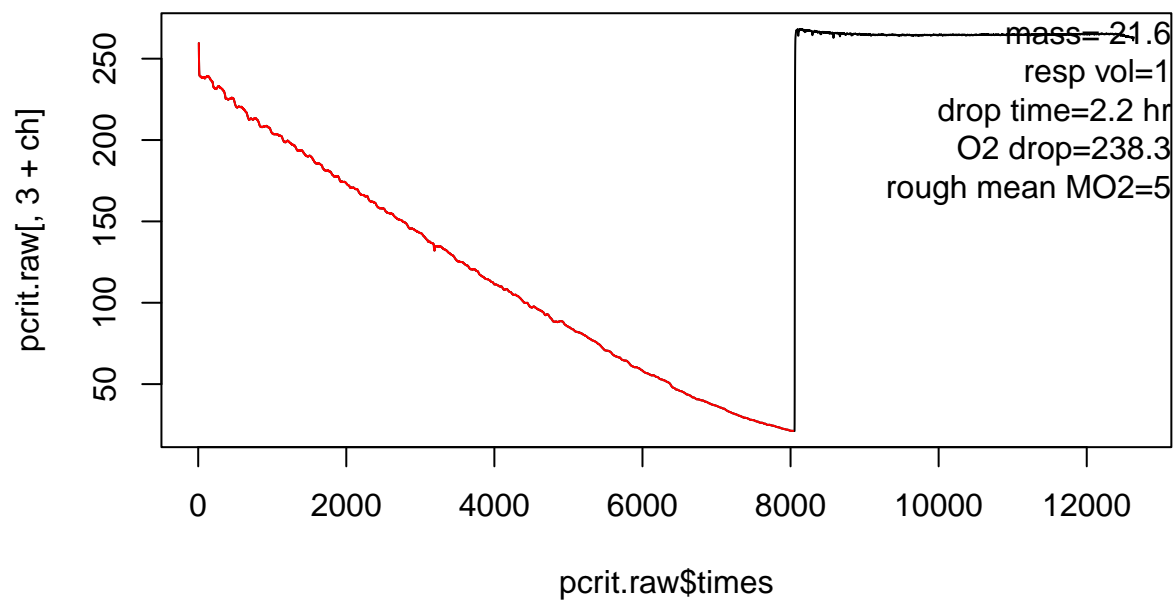
LLO @ MR of NA = NA

NLR (Power) = 40.873

Sub-PI = 3.21



Gr3 Muus 1000 pcrit 7-21-21.txt



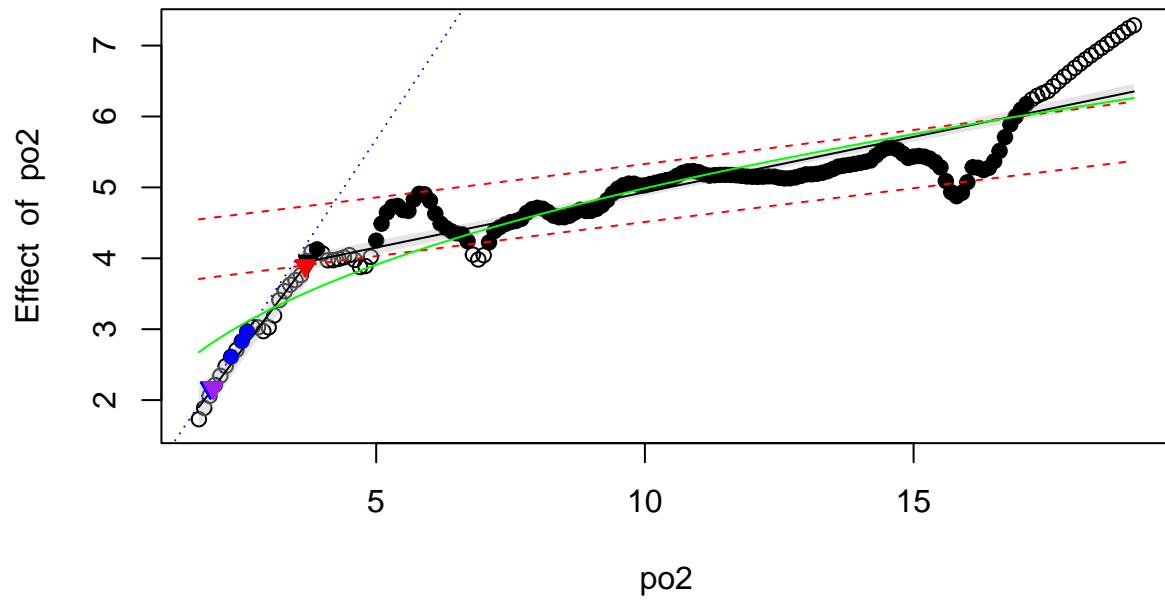
Alpha @ MR of 2.17 = 1.913

Breakpoint = 3.74

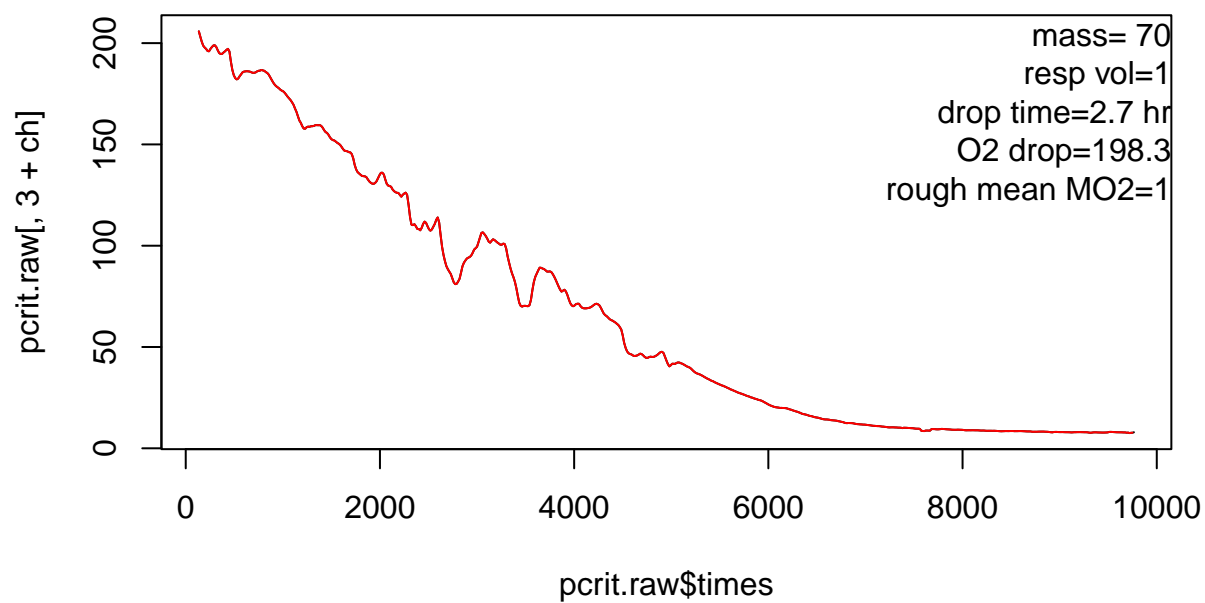
LLO @ MR of 2.17 = 1.97

NLR (Power) = 67.77

Sub-PI = 3.68



gr3 muus 1800 7day Pcrit 7-20-21.txt



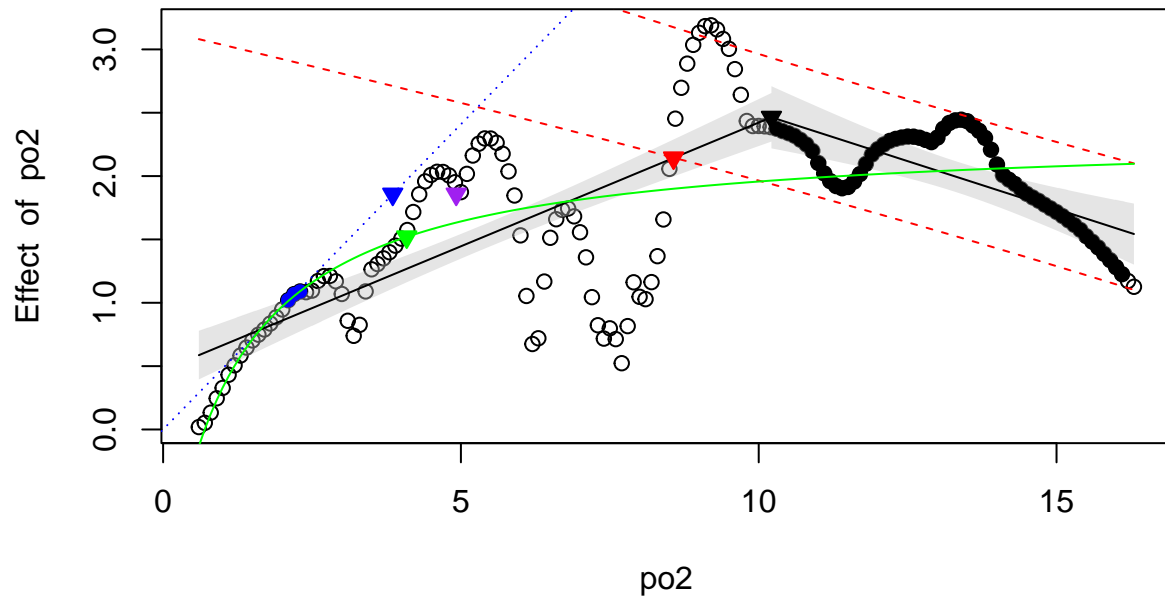
Alpha @ MR of 1.86 = 3.855

Breakpoint = 10.214

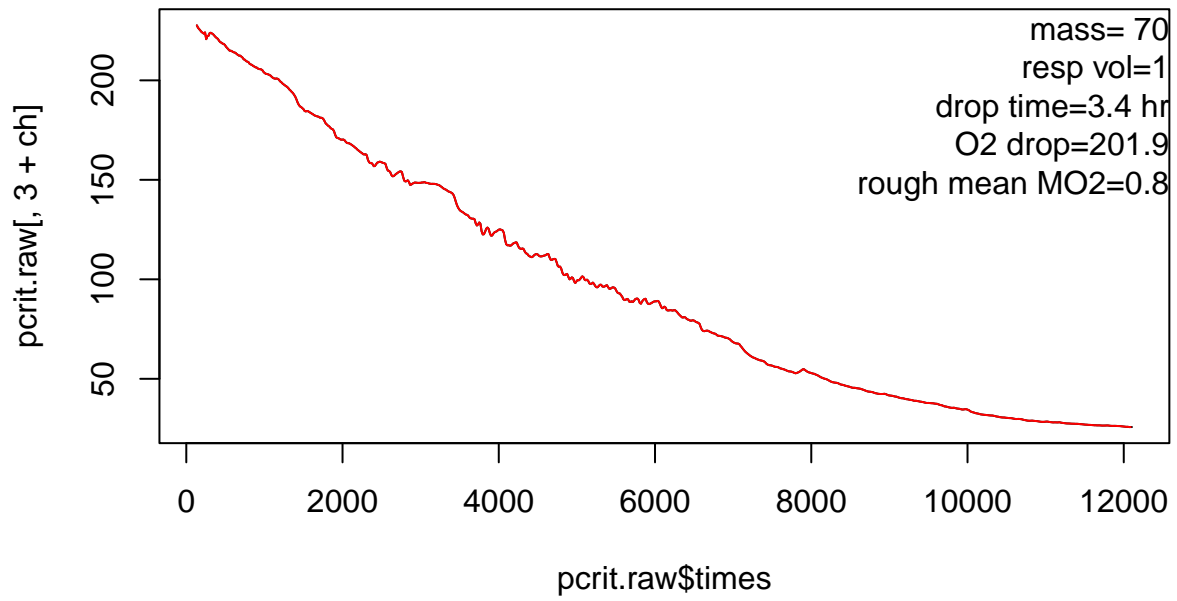
LLO @ MR of 1.86 = 4.921

NLR (Hyperbola) = 4.09

Sub-PI = 8.57



gr3 muus 1800 pcrit 7-13-21.txt



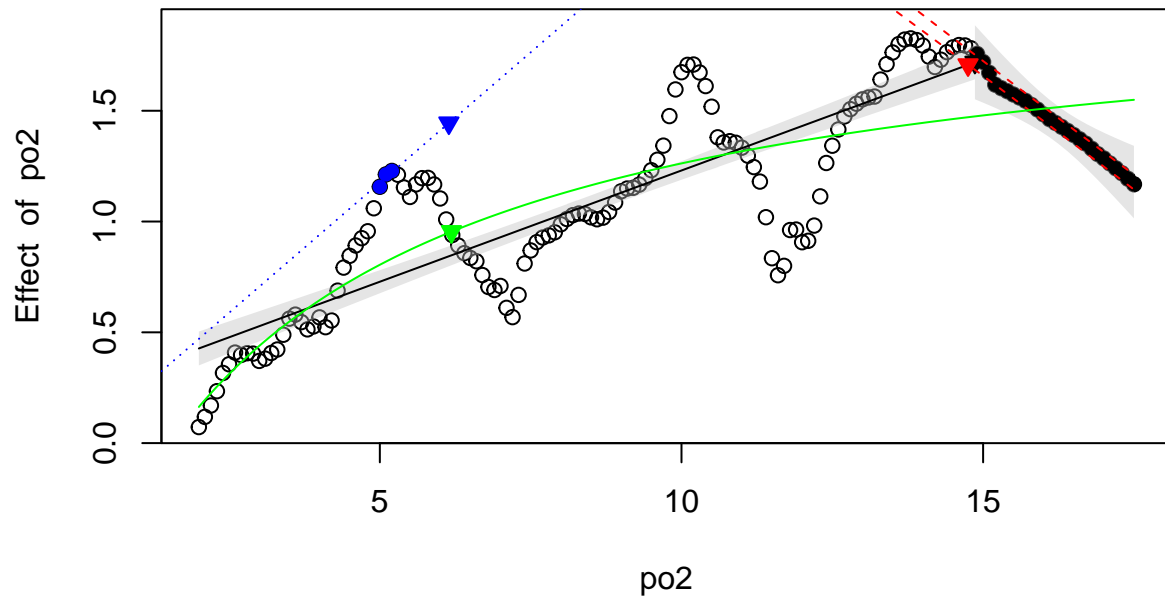
Alpha @ MR of 1.44 = 6.145

Breakpoint = 14.864

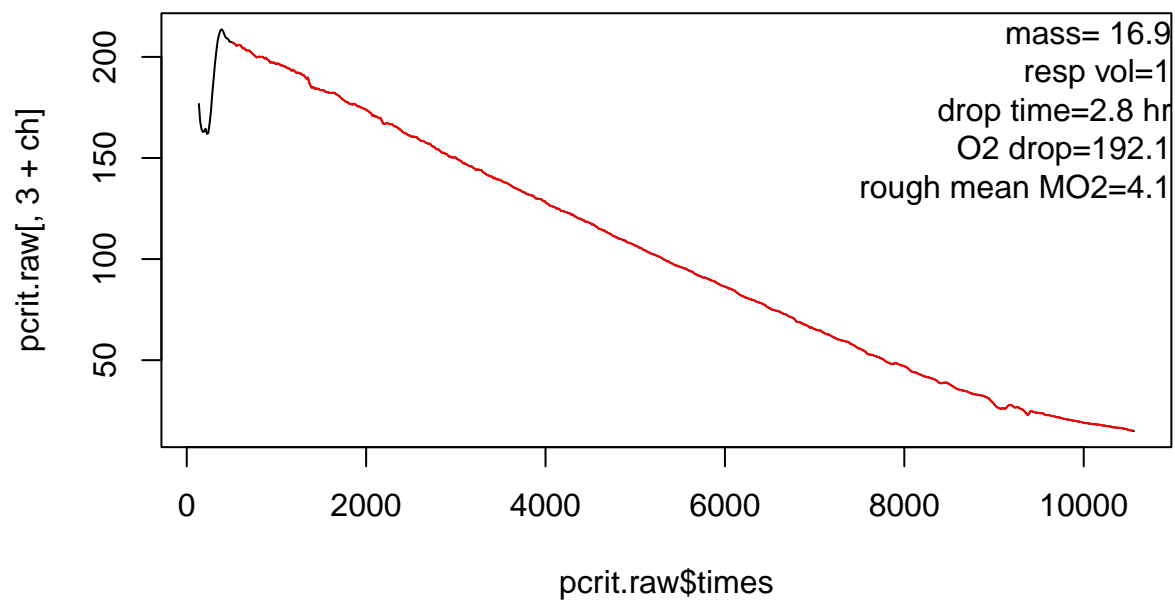
LLO @ MR of NA = NA

NLR (Hyperbola) = 6.188

Sub-PI = 14.75



Gr3 Muus 1800-2 pcrit 07-28-21.txt



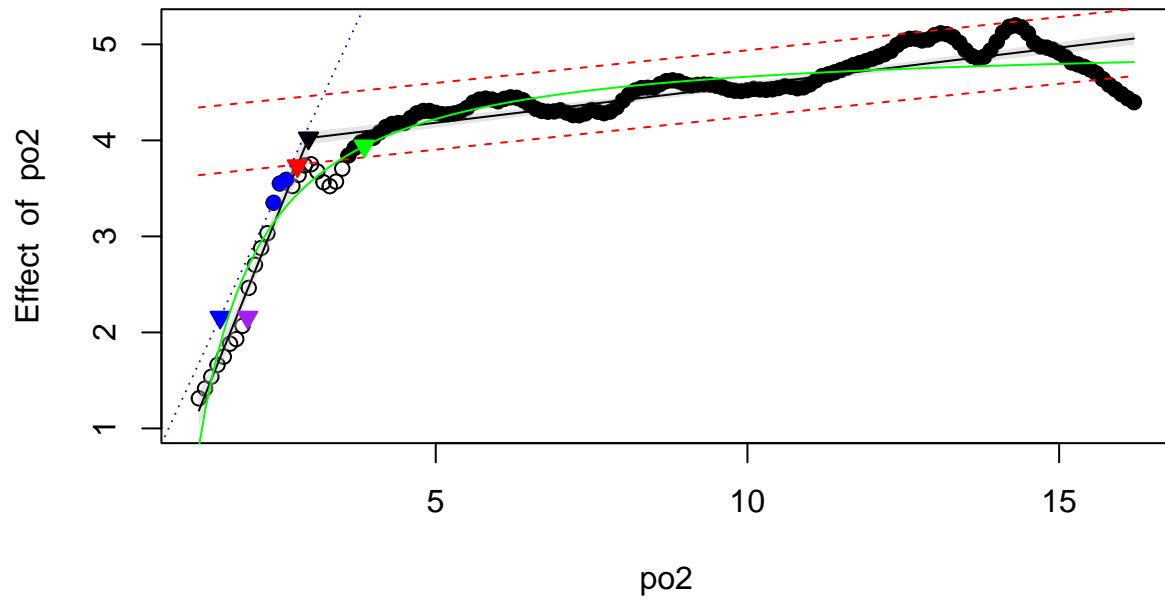
Alpha @ MR of 2.16 = 1.542

Breakpoint = 2.959

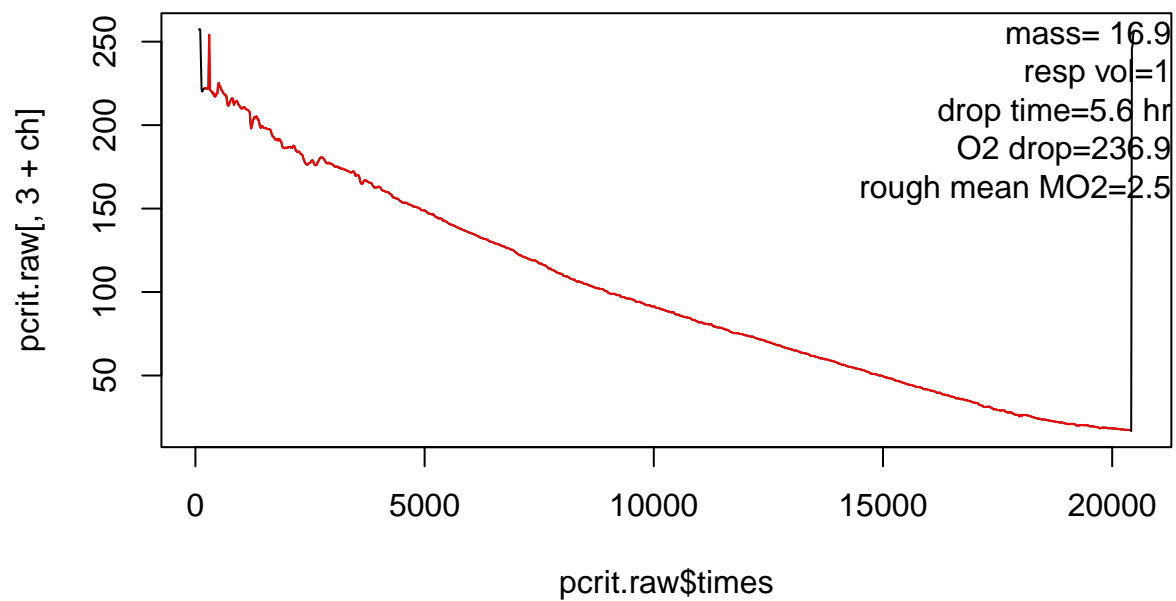
LLO @ MR of 2.16 = 1.983

NLR (Pareto) = 3.857

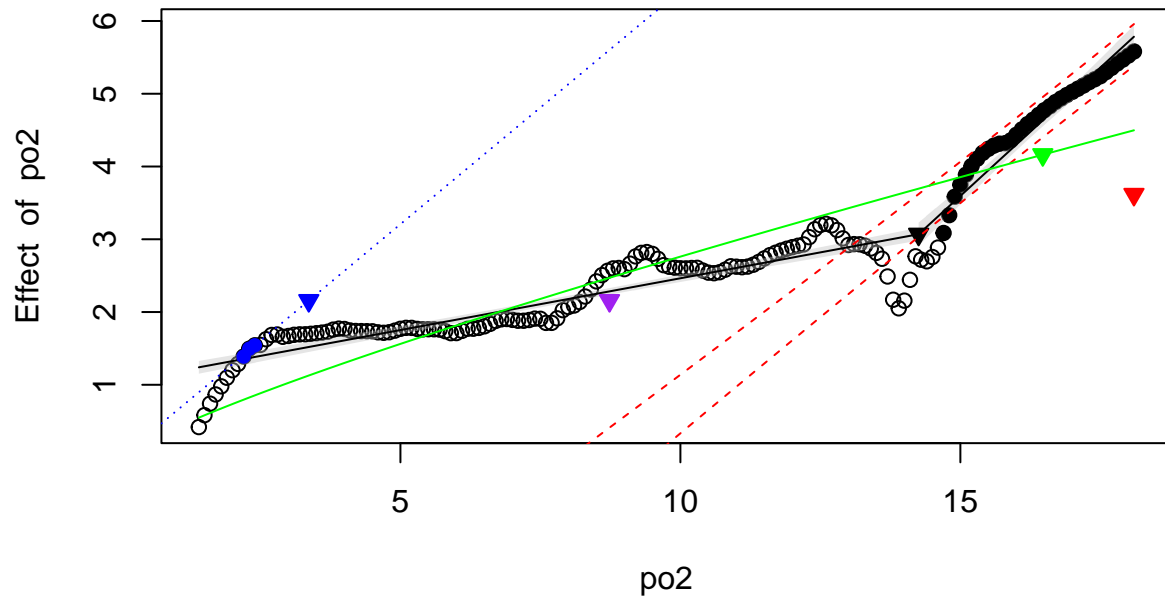
Sub-PI = 2.78



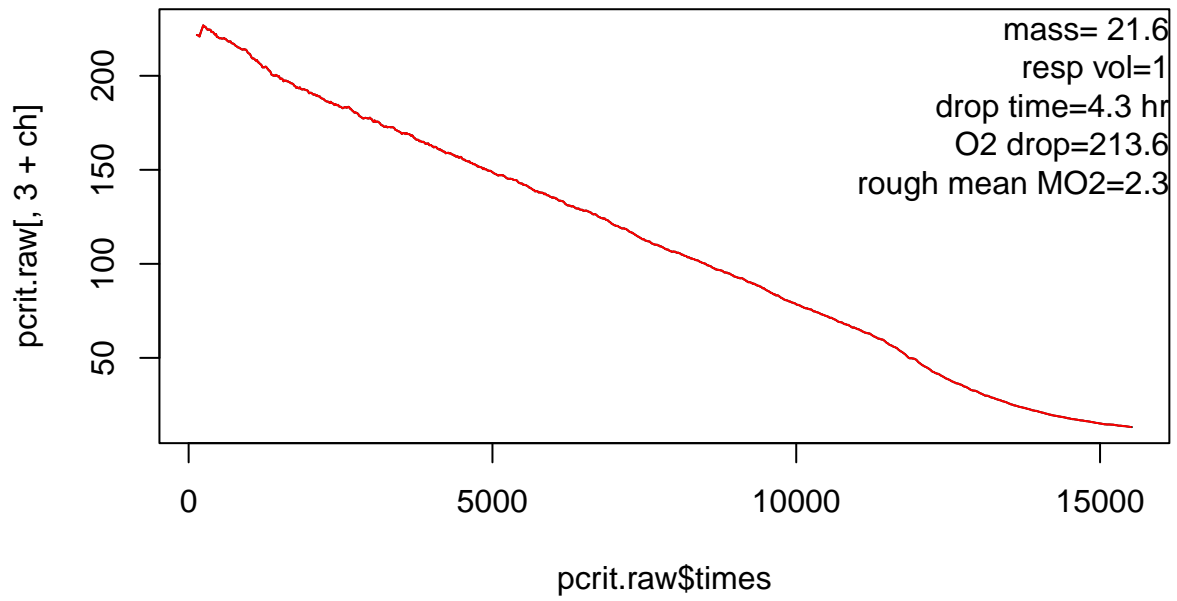
Gr3 Muus 1800-2 pcrit 08-03-21.txt



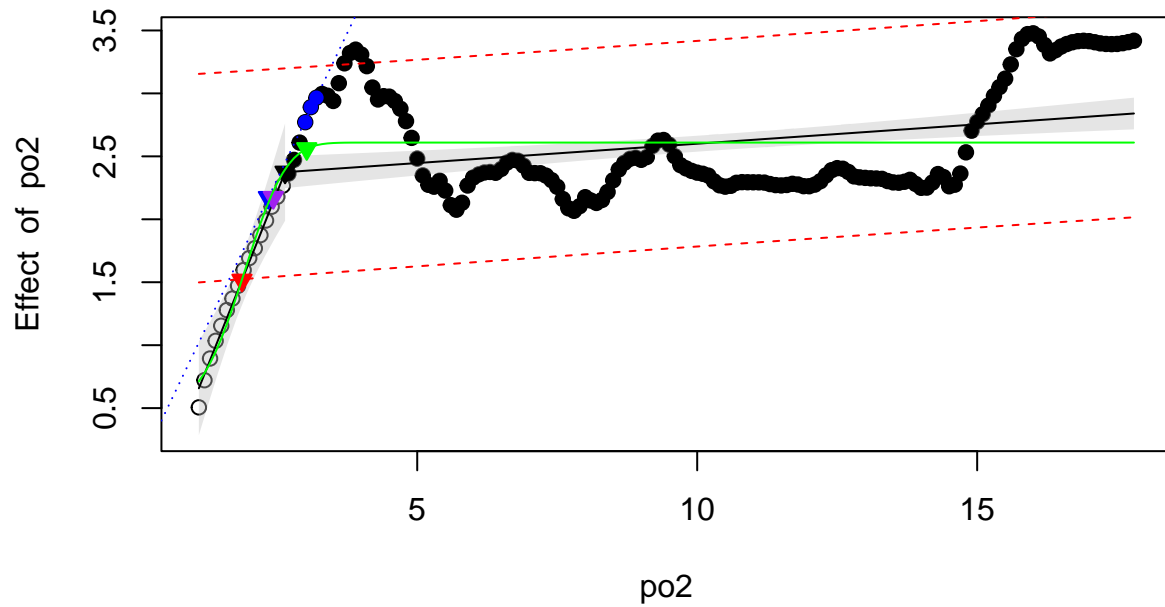
Alpha @ MR of 2.16 = 3.363
Breakpoint = 14.254
LLO @ MR of 2.16 = 8.734
NLR (Power) = 16.472
Sub-PI = 18.1



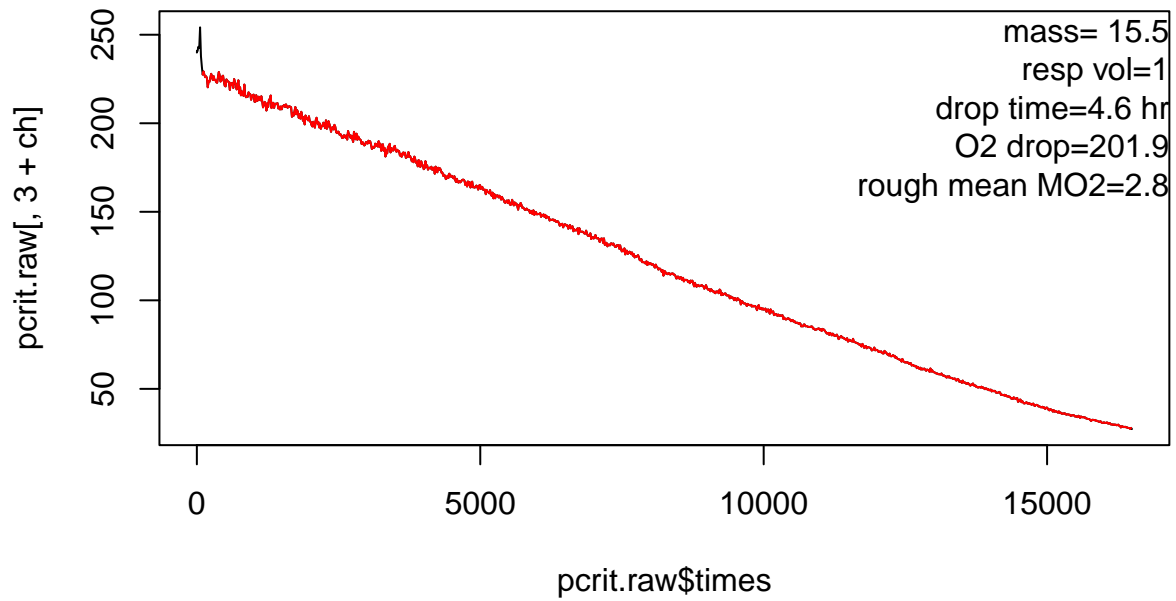
Gr3 Muus1000-2 7 day pcrit 7-27-21.txt



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



GR4MUUS1000-2Pcrit-7-26-21-ch1.txt



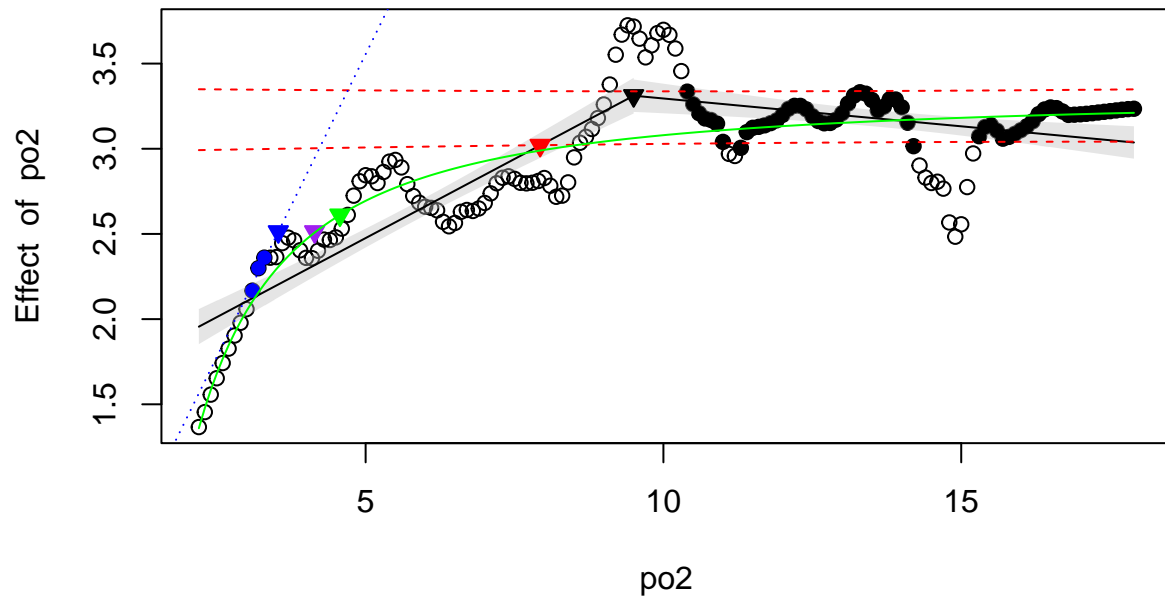
Alpha @ MR of 2.51 = 3.535

Breakpoint = 9.5

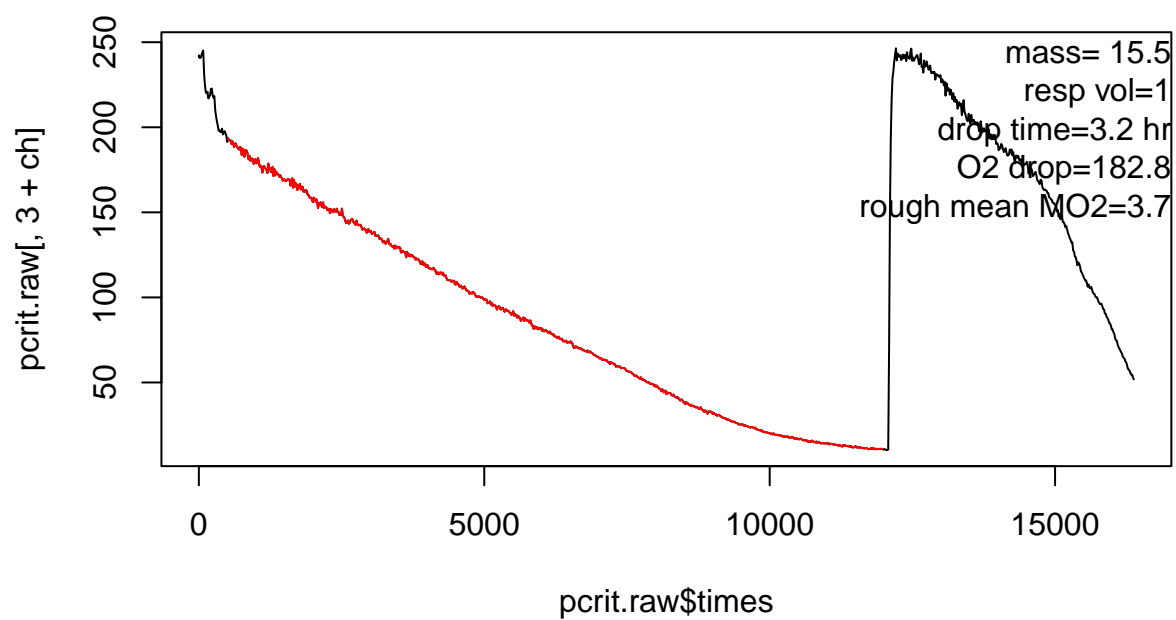
LLO @ MR of 2.51 = 4.144

NLR (Pareto) = 4.569

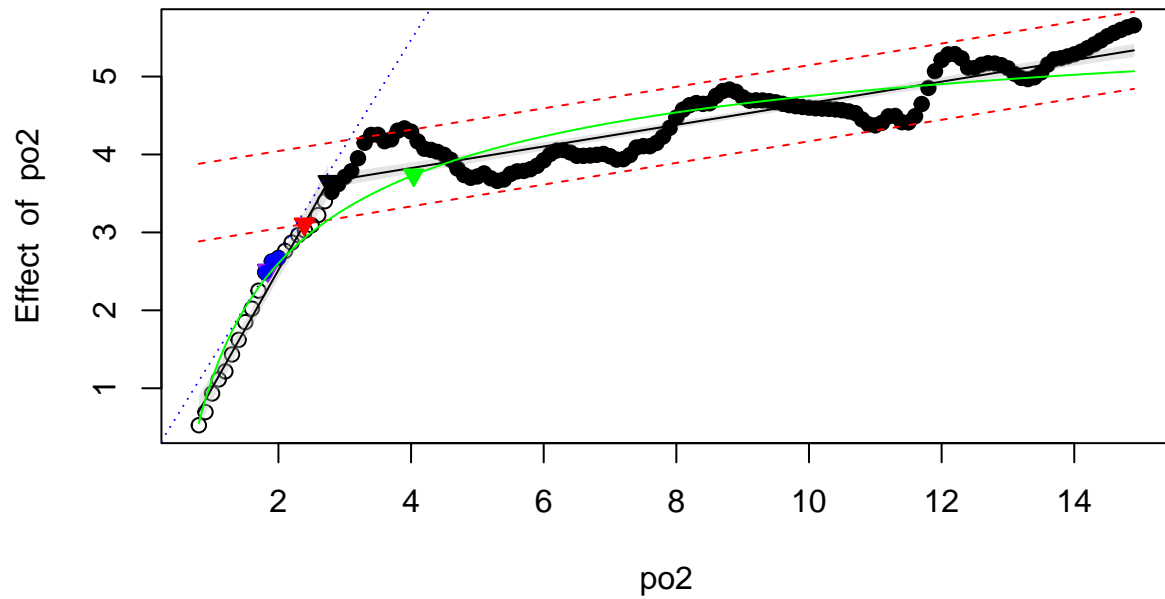
Sub-PI = 7.93



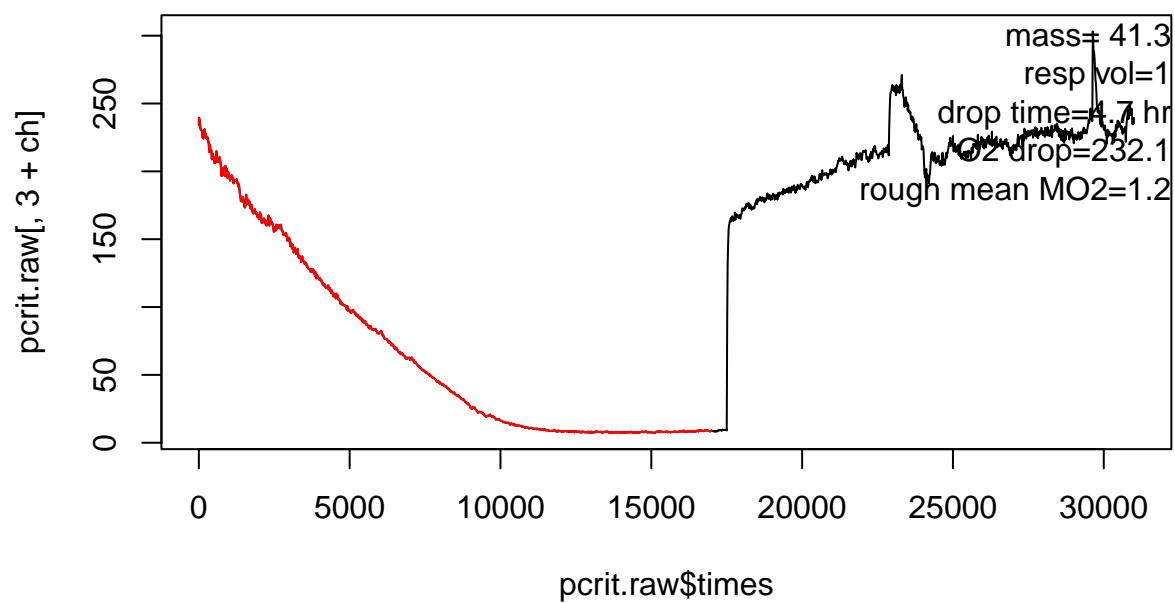
GR4MUUS1000Pcrit-7-21-21-ch1.txt



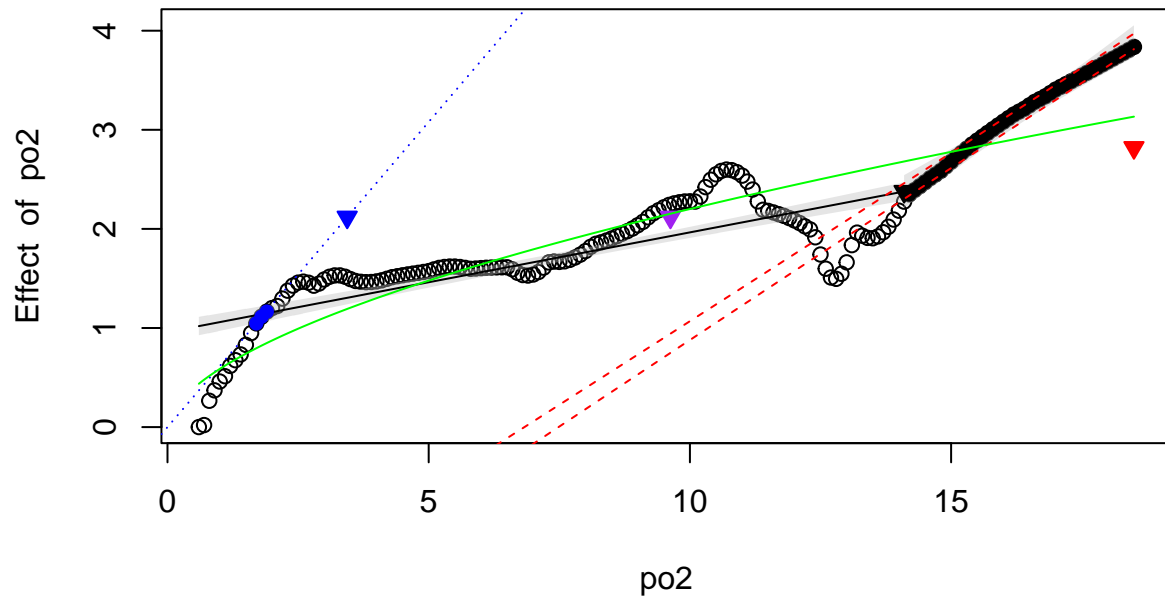
Alpha @ MR of 2.51 = 1.838
Breakpoint = 2.75
LLO @ MR of 2.51 = 1.843
NLR (Weibull with intercept) = 4.04
Sub-PI = 2.39



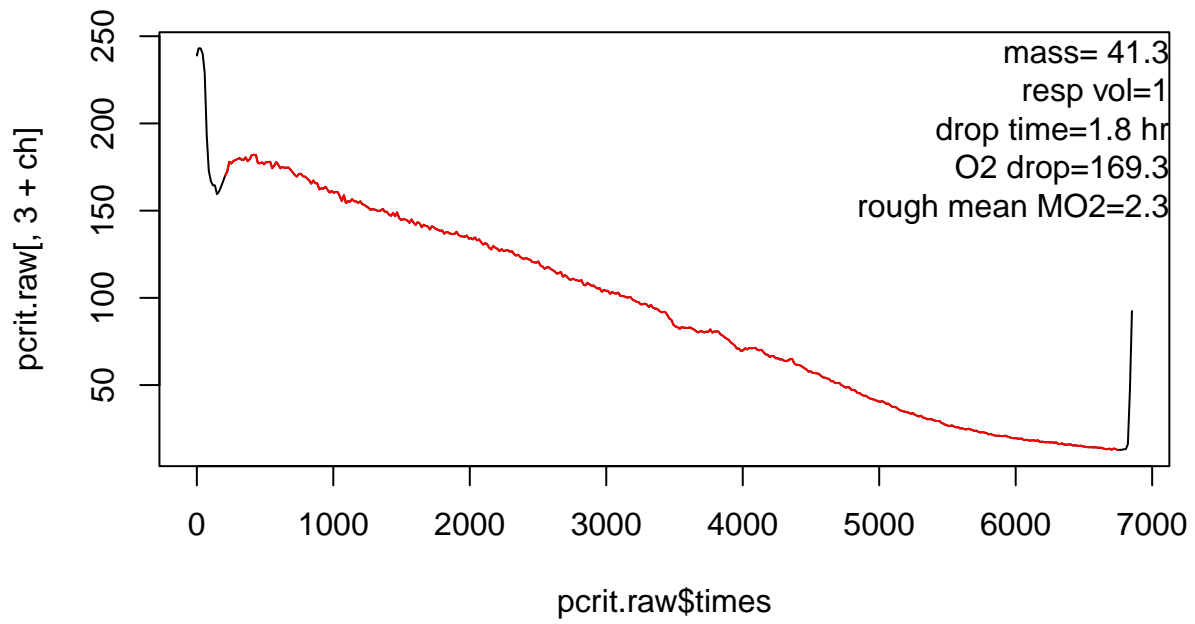
GR4MUUS1800-2-7dayPcrit-8-3-21-ch1.txt



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



GR4MUUS1800-2Pcrit-7-28-21-ch1.txt



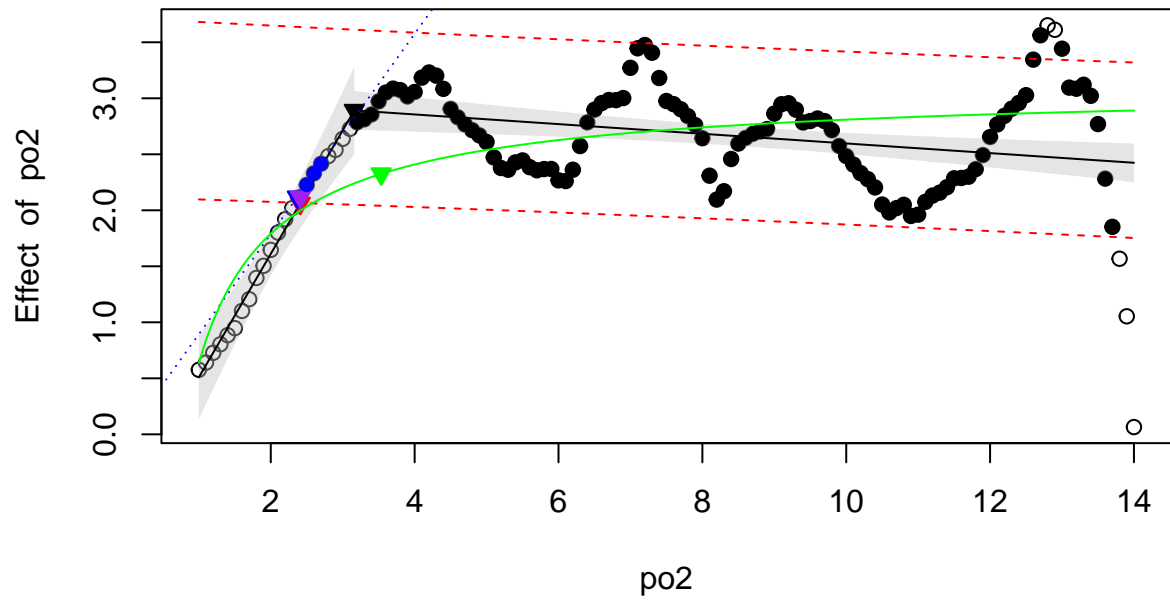
Alpha @ MR of 2.12 = 2.37

Breakpoint = 3.162

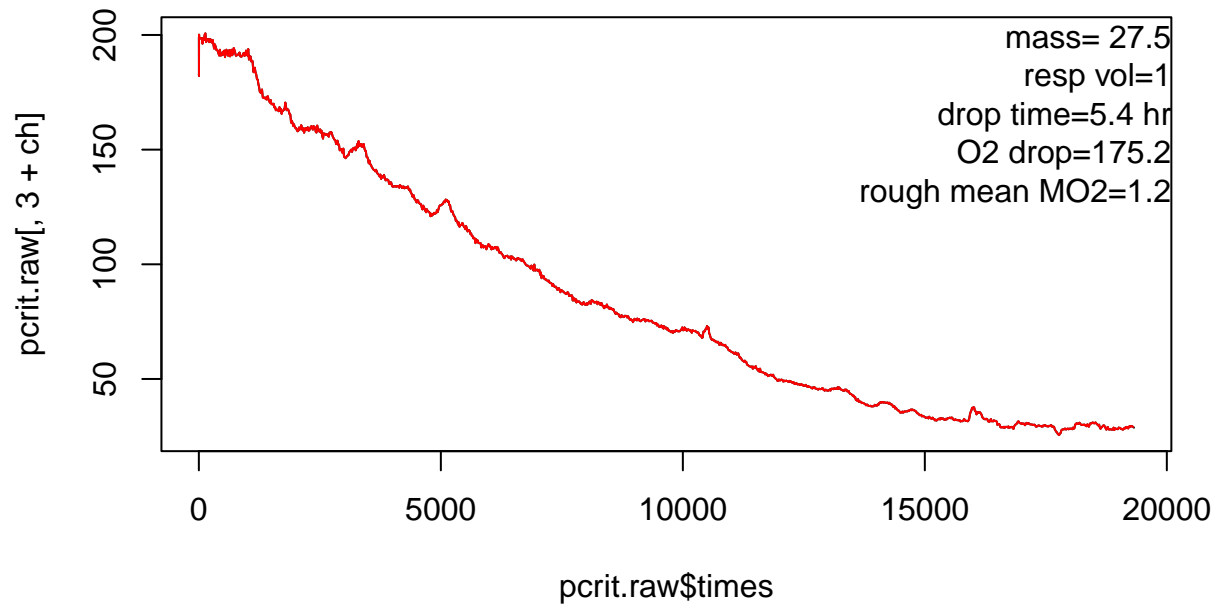
LLO @ MR of 2.12 = 2.413

NLR (Pareto) = 3.535

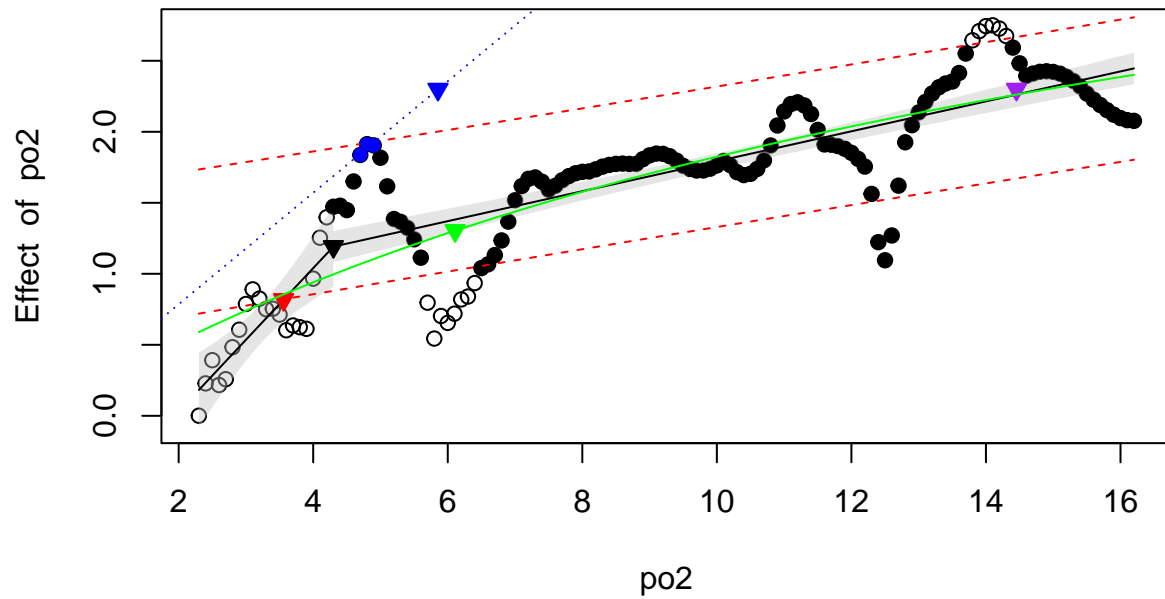
Sub-PI = 2.41



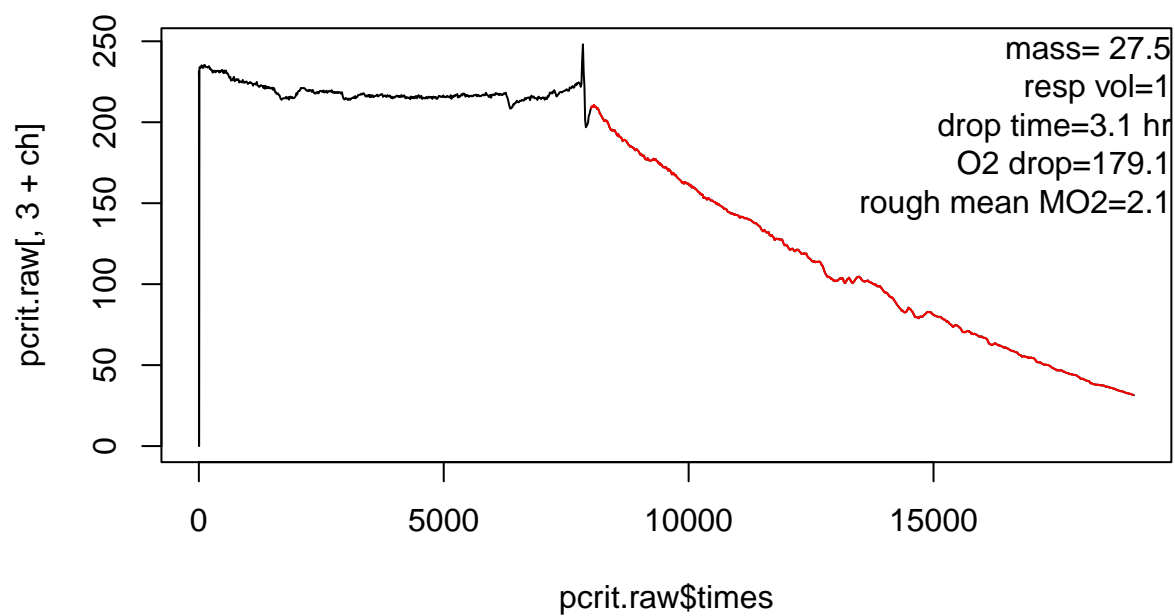
GR4MUUS1800-7dayPcrit-7-20-21-ch1.txt



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



GR4MUUS1800Pcrit-7-13-21-ch1.txt



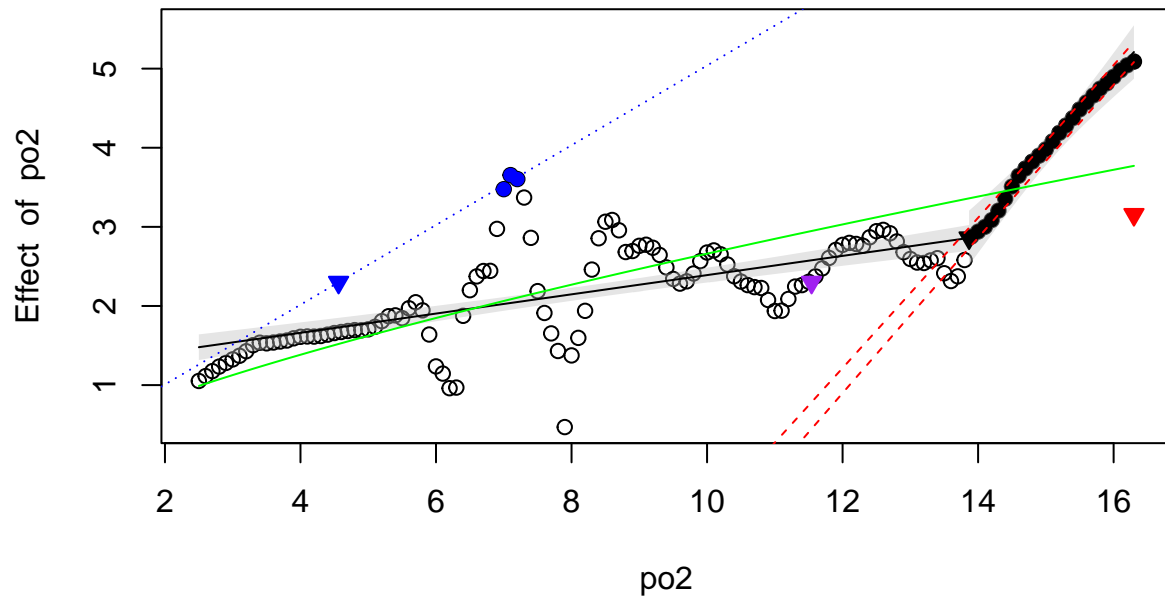
Alpha @ MR of 2.3 = 4.562

Breakpoint = 13.867

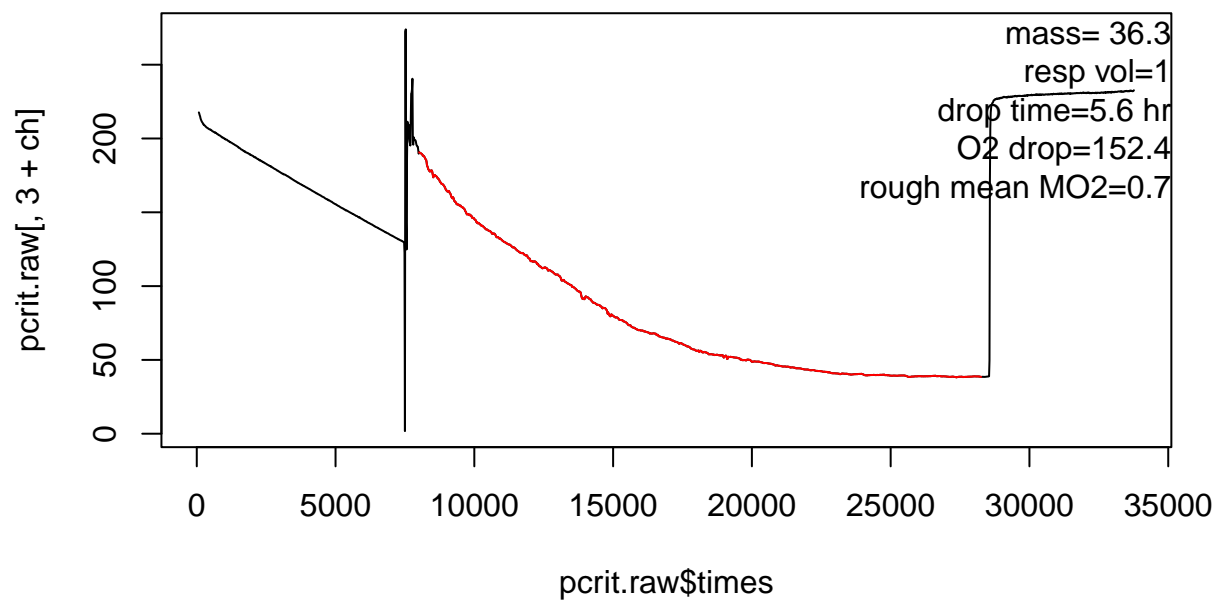
LLO @ MR of 2.3 = 11.542

NLR (Power) = 37.94

Sub-PI = 16.3



tbocto 1000 pcrit tank 1 and 2 day 7 8-19-21.txt



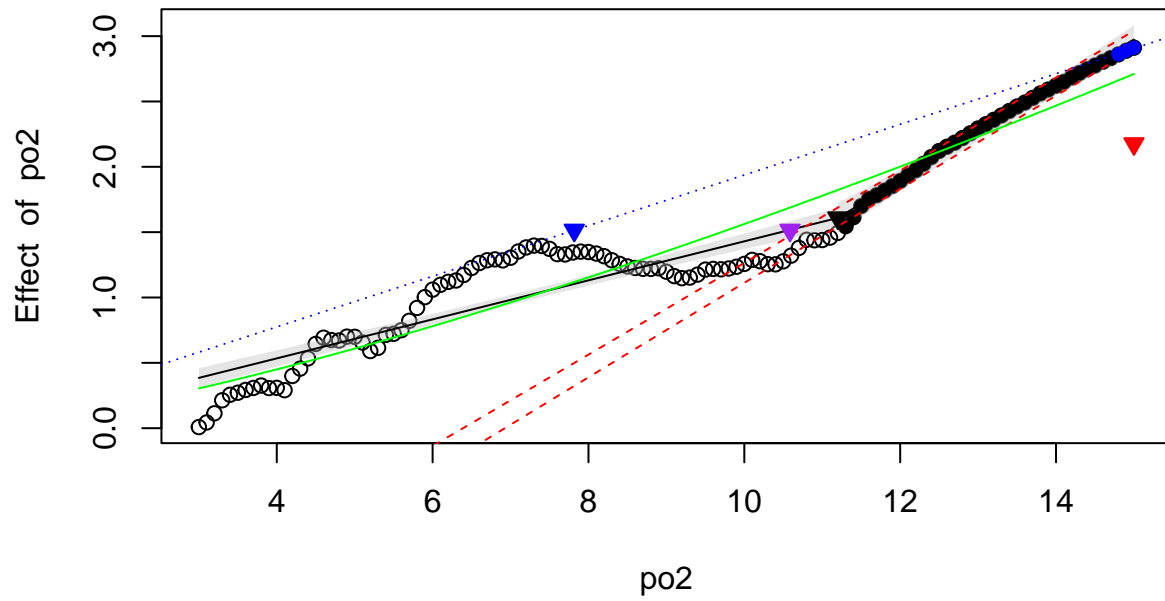
Alpha @ MR of 1.51 = 7.818

Breakpoint = 11.2

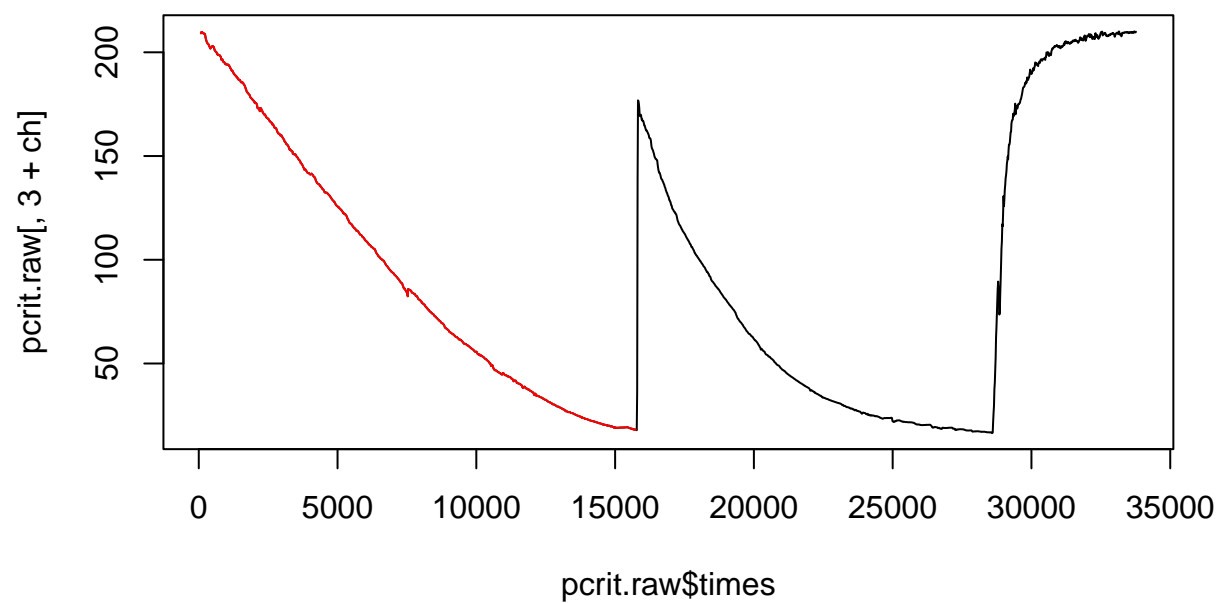
LLO @ MR of 1.51 = 10.587

NLR (Power) = 28.052

Sub-PI = 15



tbocto 1000 pcrit tank 1 and 2 day 7 8–19–21.txt



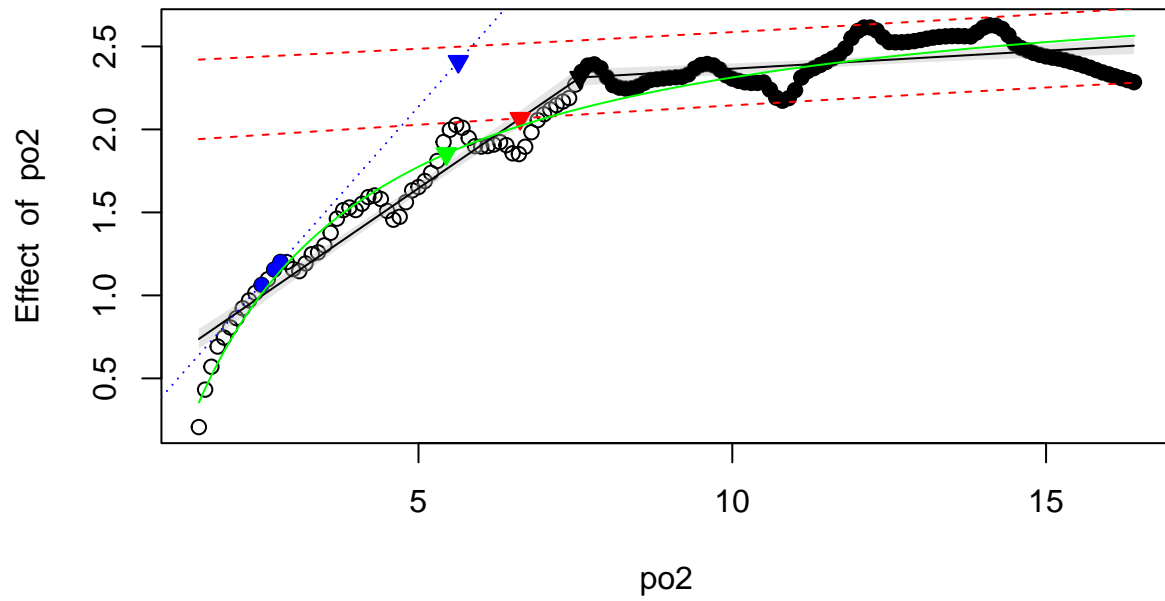
Alpha @ MR of 2.41 = 5.635

Breakpoint = 7.572

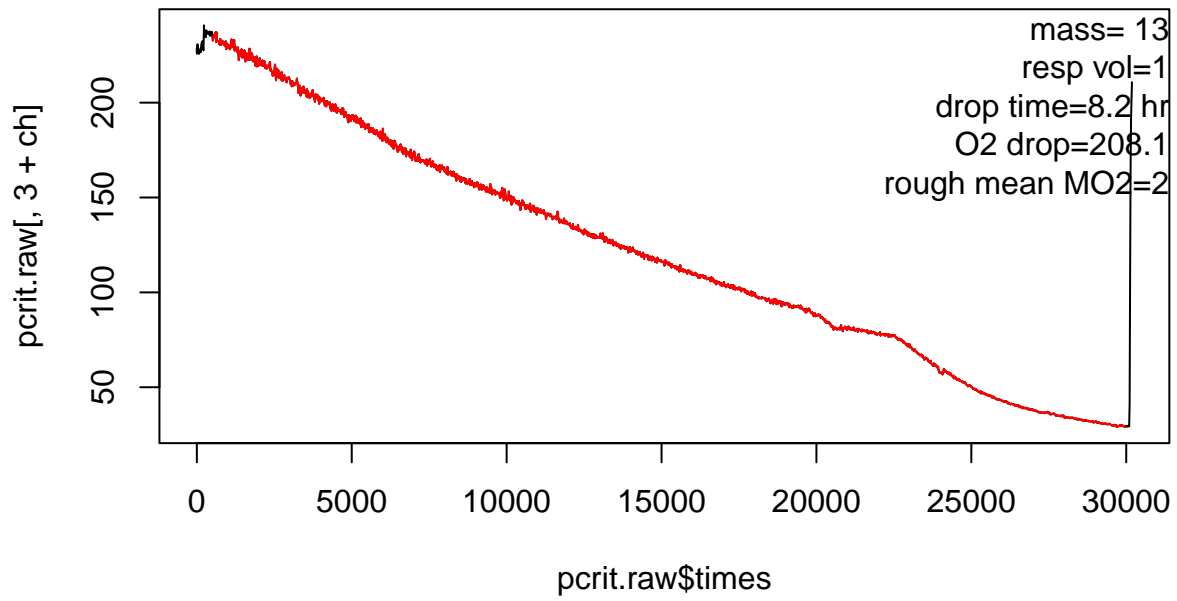
LLO @ MR of NA = NA

NLR (Hyperbola) = 5.444

Sub-PI = 6.62



tbocto 1000 pcrit tank 3 and 4 8-11-21-ch1.txt



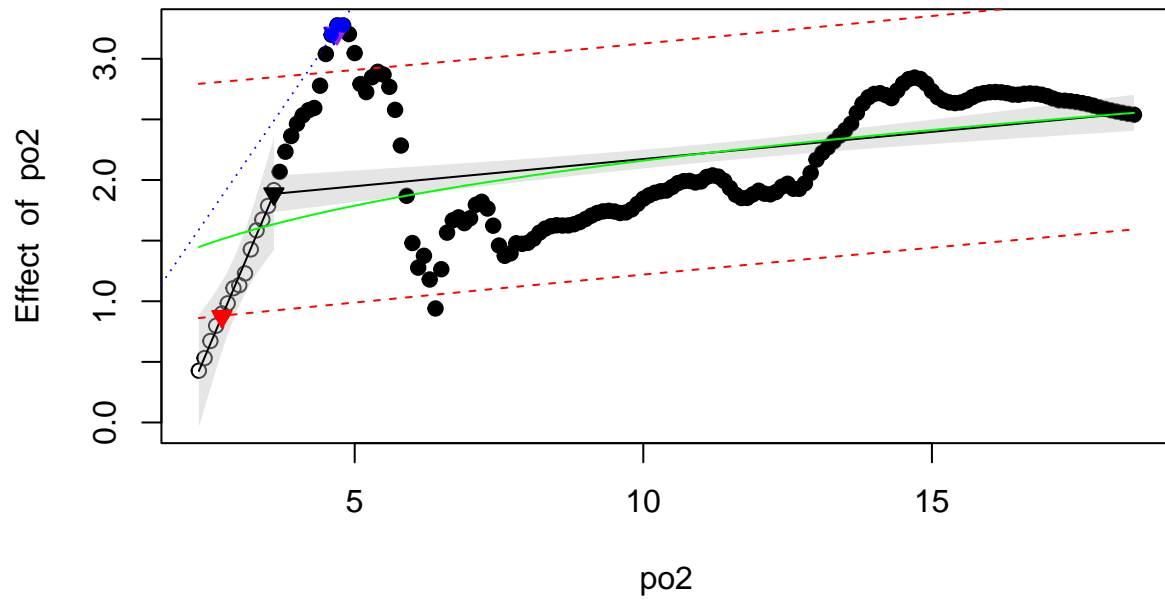
Alpha @ MR of 3.21 = 4.642

Breakpoint = 3.601

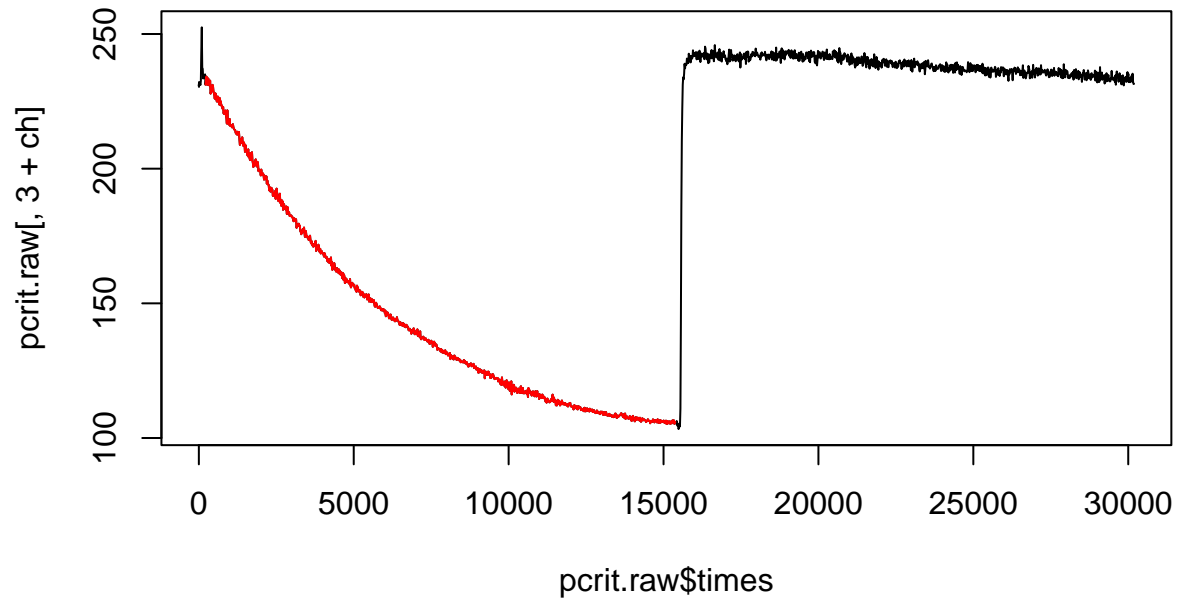
LLO @ MR of 3.21 = 4.695

NLR (Power) = 77.005

Sub-PI = 2.7



tbocto 1000 pcrit tank 3 and 4 8-11-21-ch1.txt



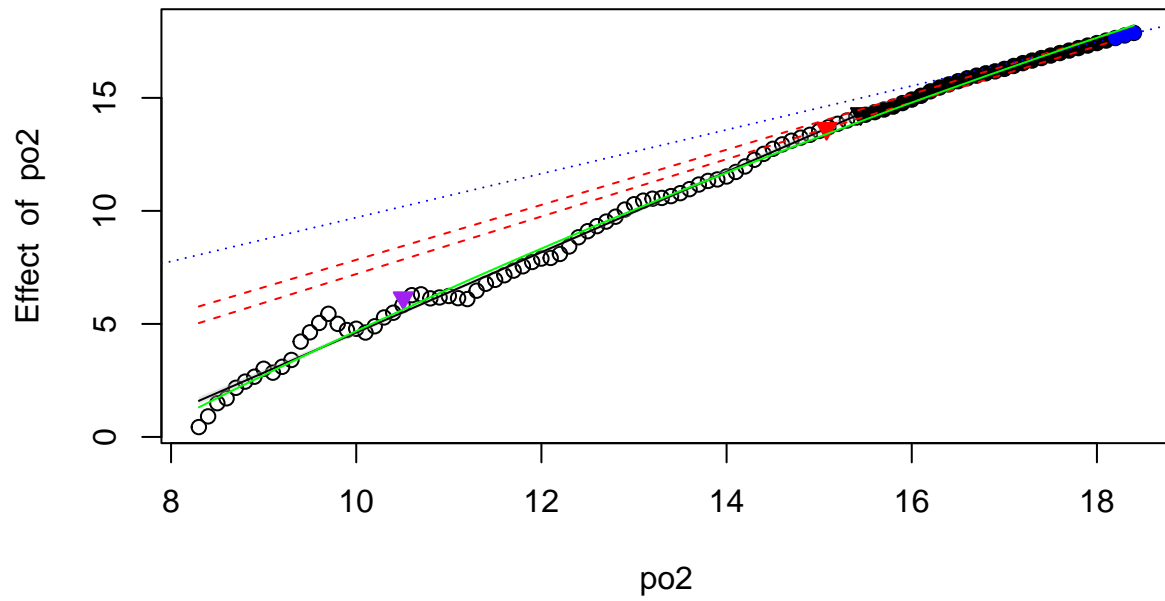
Alpha @ MR of 6.15 = 6.337

Breakpoint = 15.447

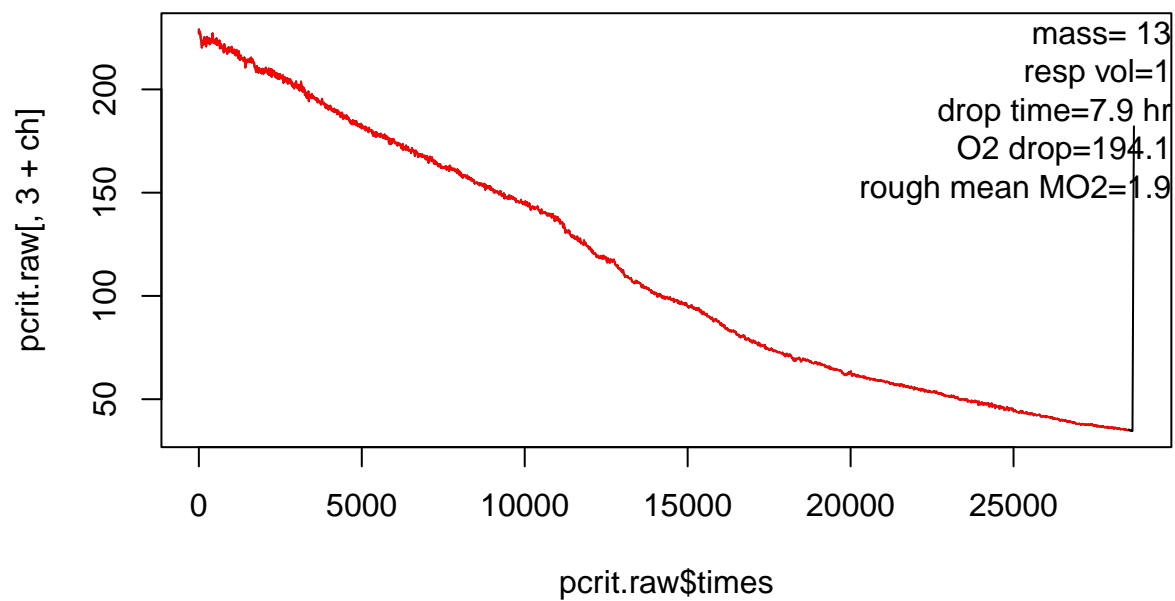
LLO @ MR of 6.15 = 10.509

NLR (Hyperbola) = 24.757

Sub-PI = 15.08



tbocto 1000 pcrit tank 3 and 4 day 7 8-19-21-ch1.txt



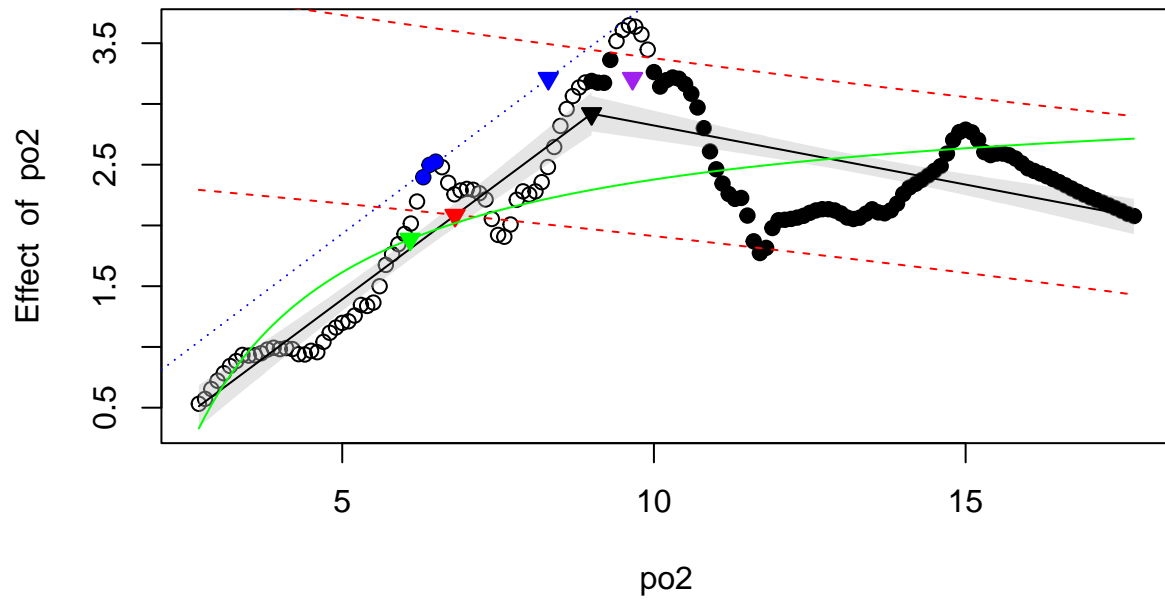
Alpha @ MR of 3.21 = 8.306

Breakpoint = 9

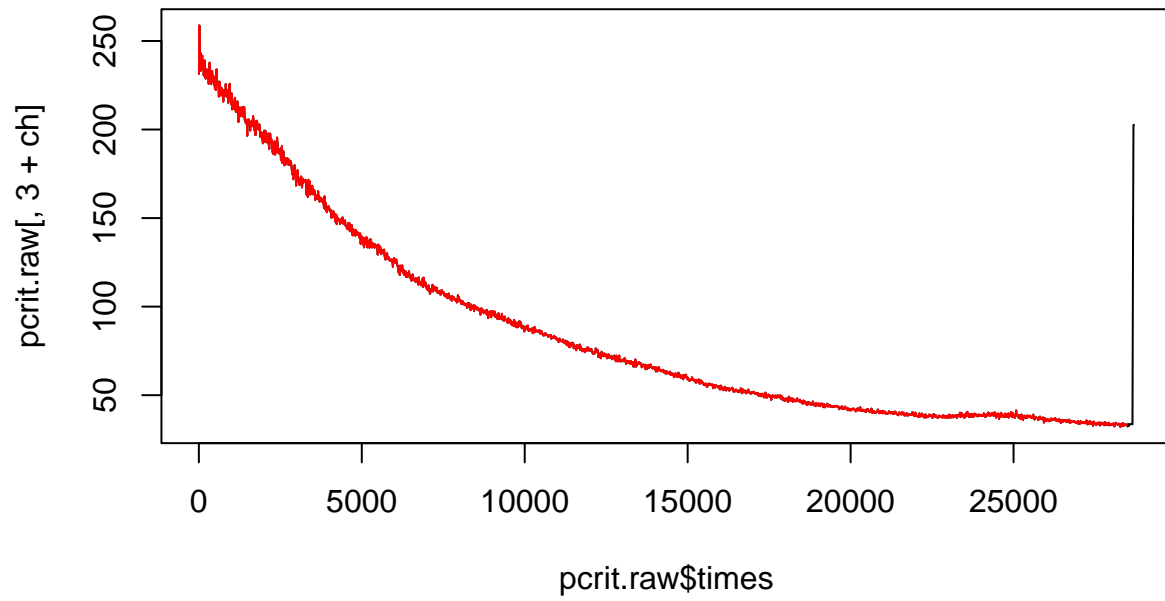
LLO @ MR of 3.21 = 9.657

NLR (Pareto) = 6.088

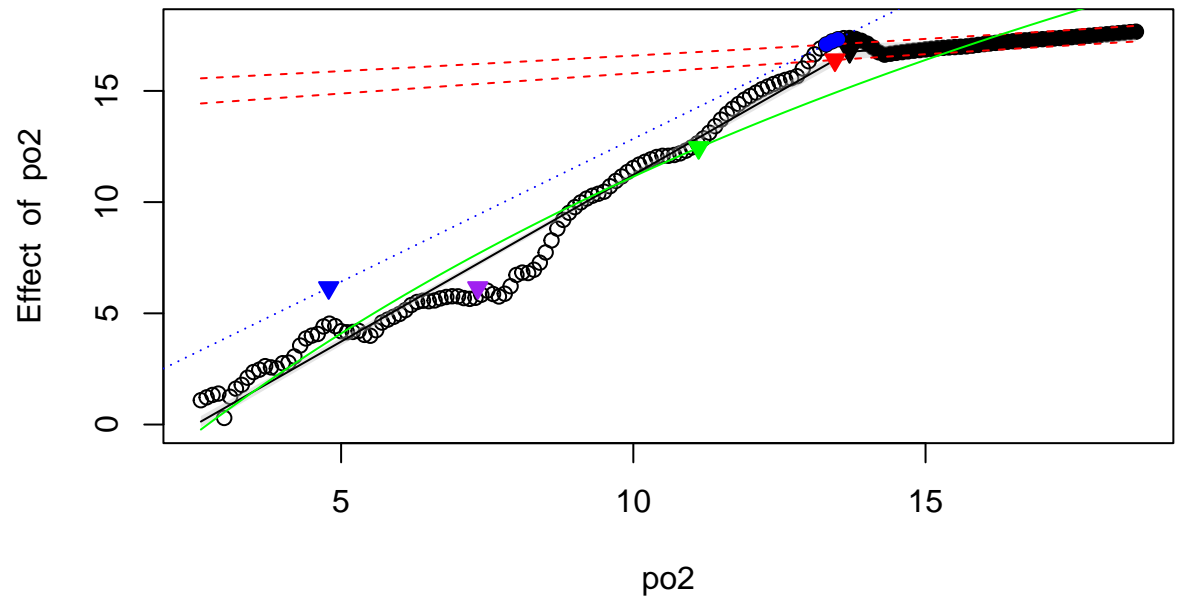
Sub-PI = 6.81



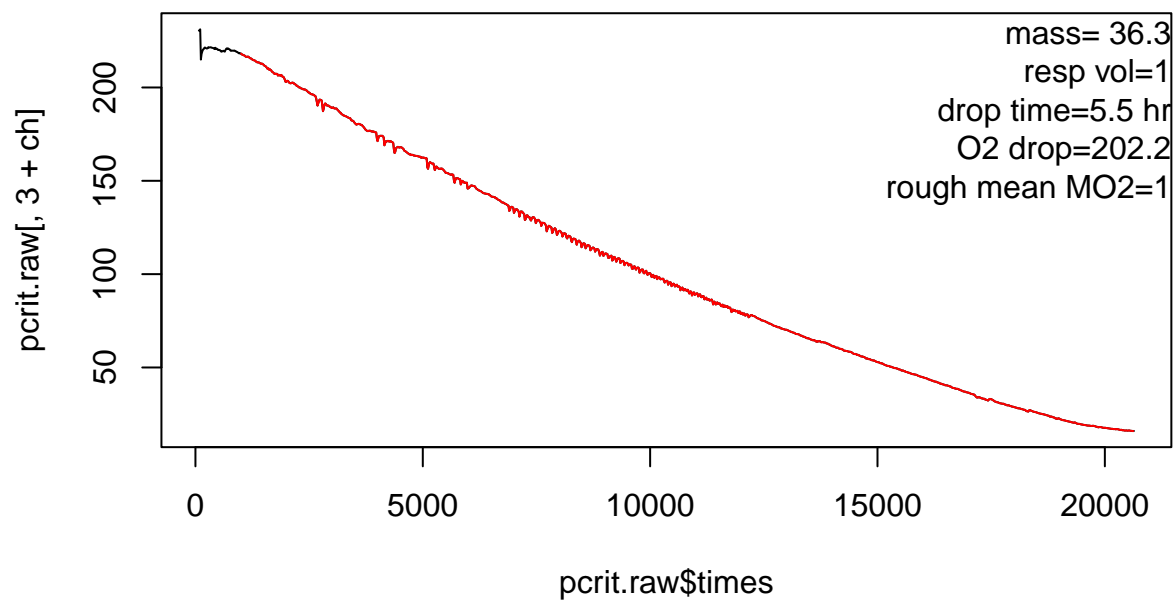
tbocto 1000 pcrit tank 3 and 4 day 7 8-19-21-ch1.txt



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



Tbocto 1000 pcrti tank 1 and 2 8-11-21.txt



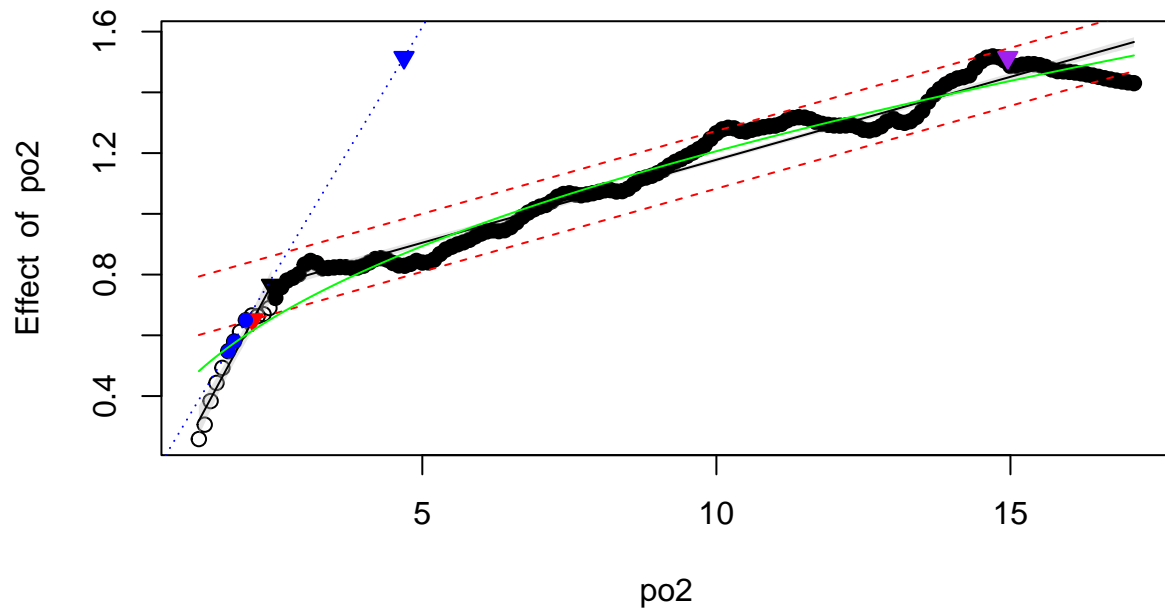
Alpha @ MR of 1.51 = 4.689

Breakpoint = 2.435

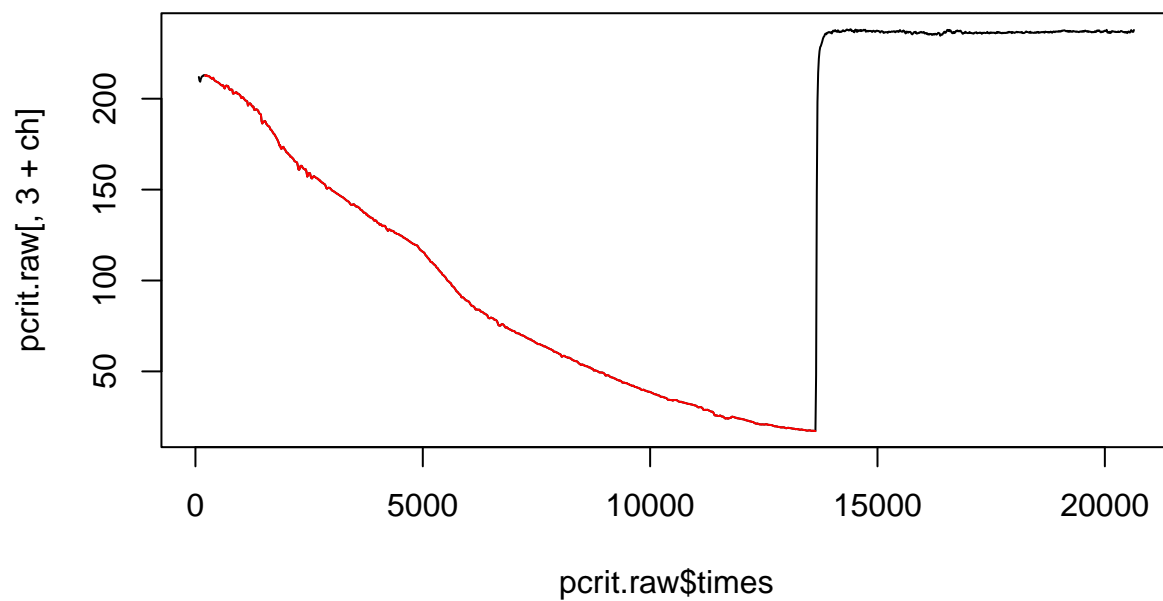
LLO @ MR of 1.51 = 14.954

NLR (Power) = 66.218

Sub-PI = 2.12



Tbocto 1000 pcrti tank 1 and 2 8-11-21.txt



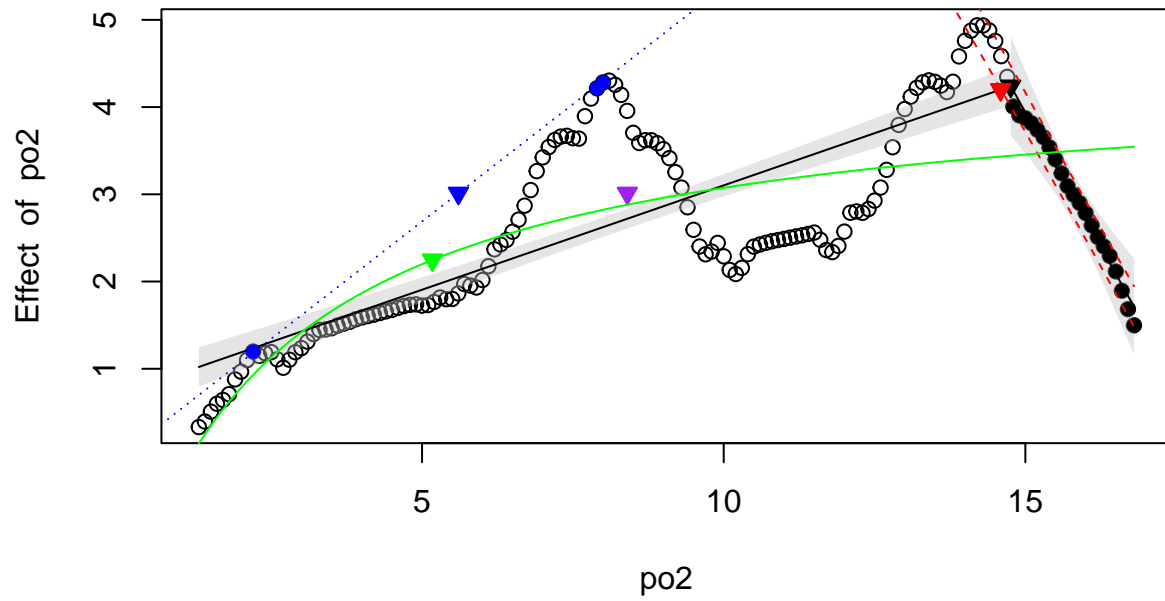
Alpha @ MR of 3.01 = 5.603

Breakpoint = 14.758

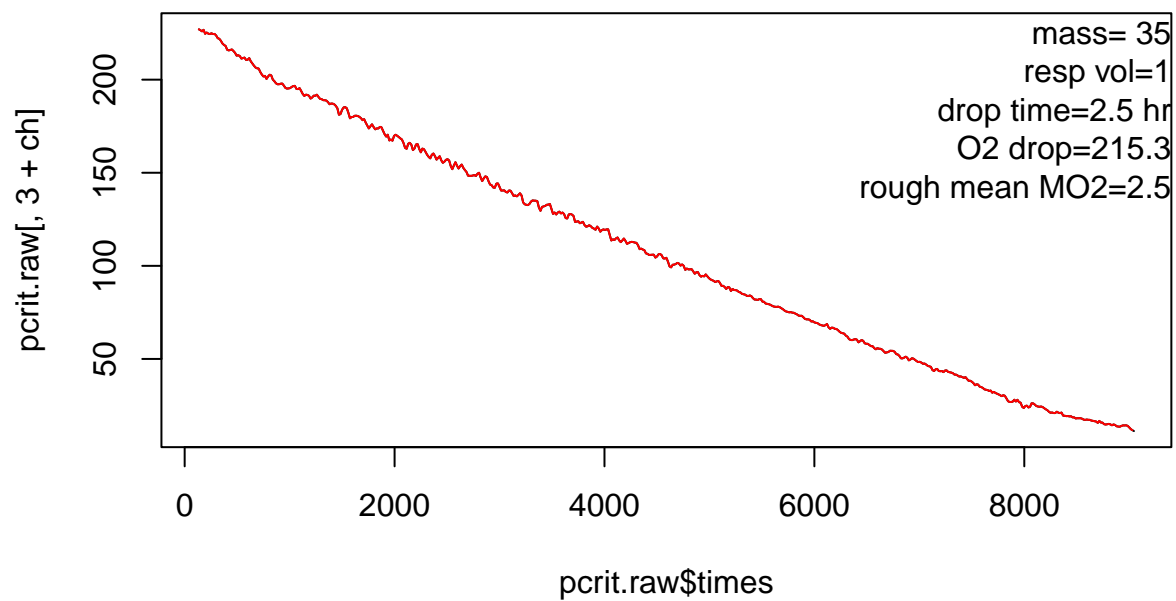
LLO @ MR of 3.01 = 8.403

NLR (Hyperbola) = 5.171

Sub-PI = 14.59



gr2muus1000 pcrit 7-21-21.txt



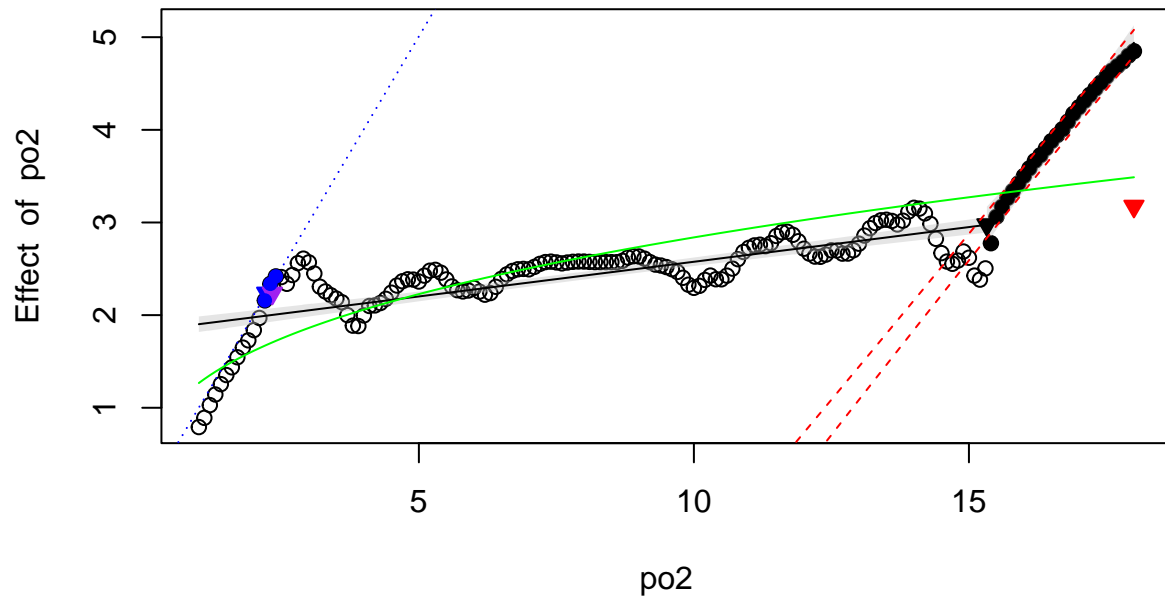
Alpha @ MR of 2.24 = 2.233

Breakpoint = 15.327

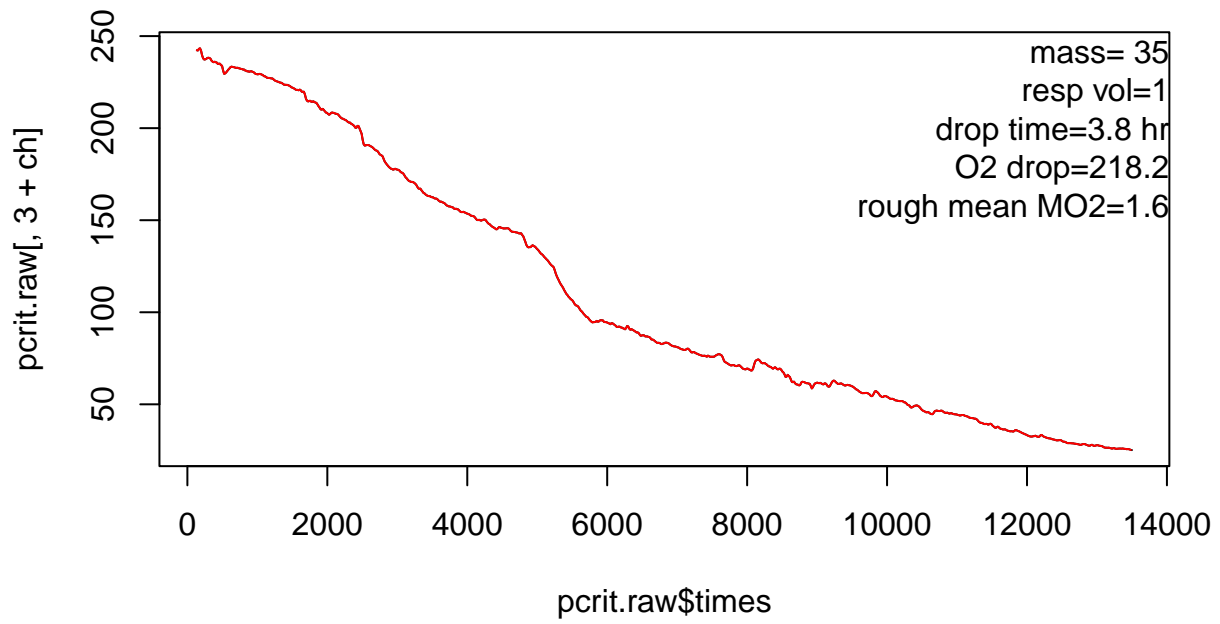
LLO @ MR of 2.24 = 2.337

NLR (Power) = 64.181

Sub-PI = 18



gr2muus1000-2 pcrit 7-26-21.txt



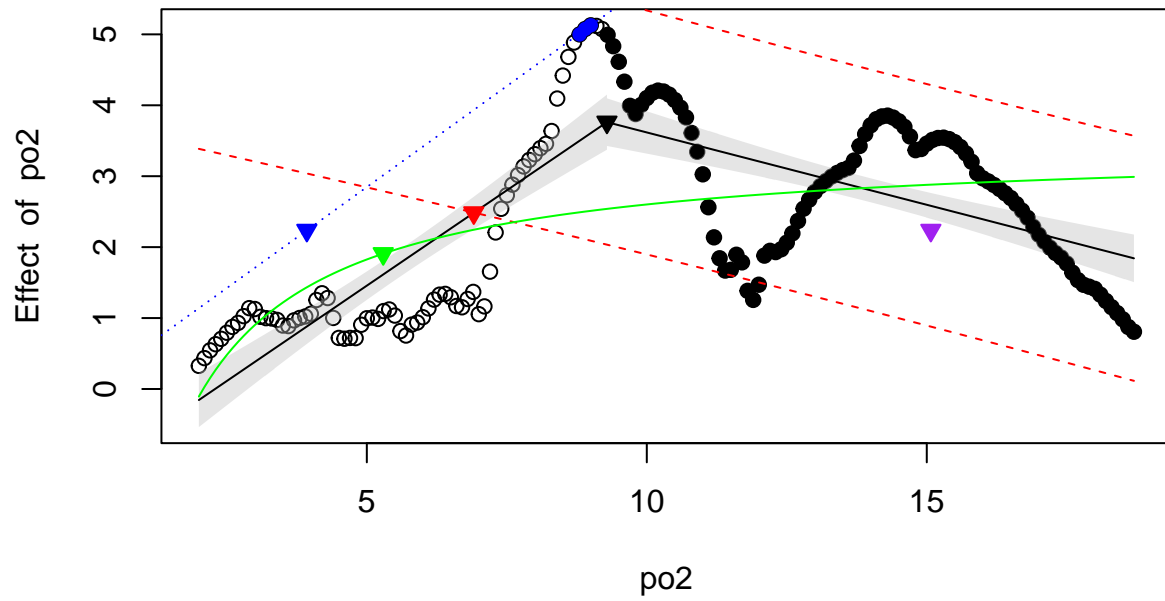
Alpha @ MR of 2.24 = 3.929

Breakpoint = 9.288

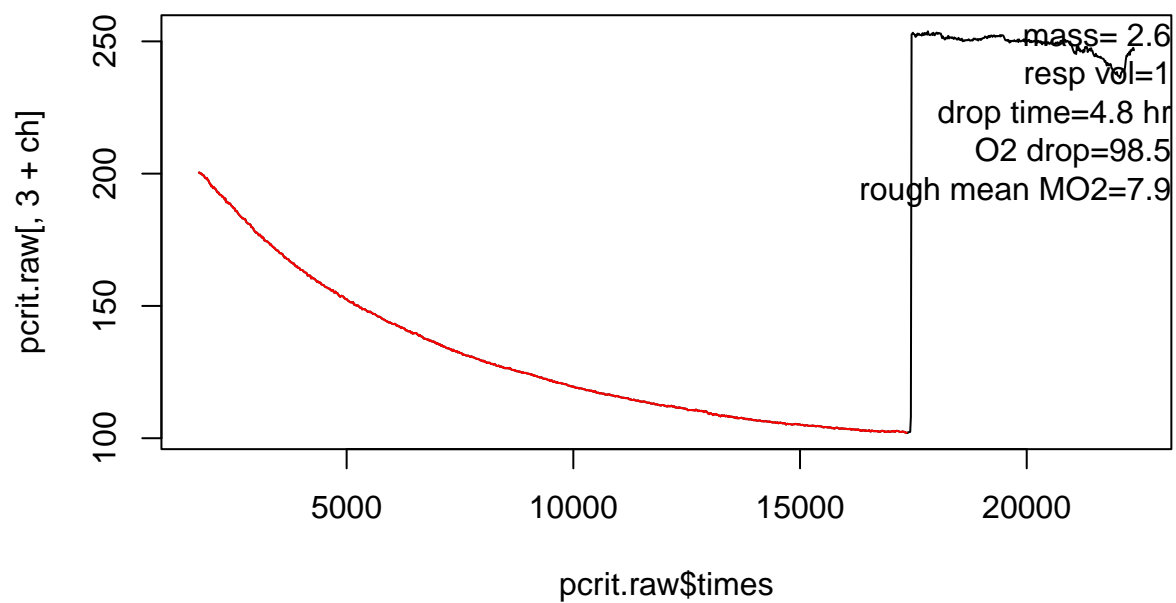
LLO @ MR of 2.24 = 15.073

NLR (Hyperbola) = 5.293

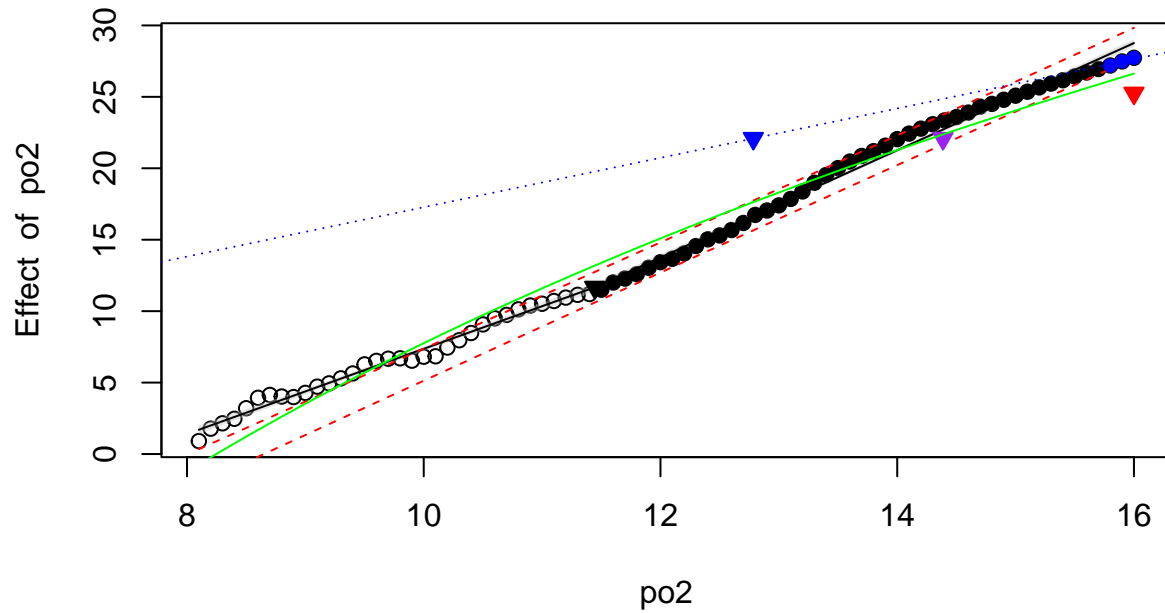
Sub-PI = 6.91



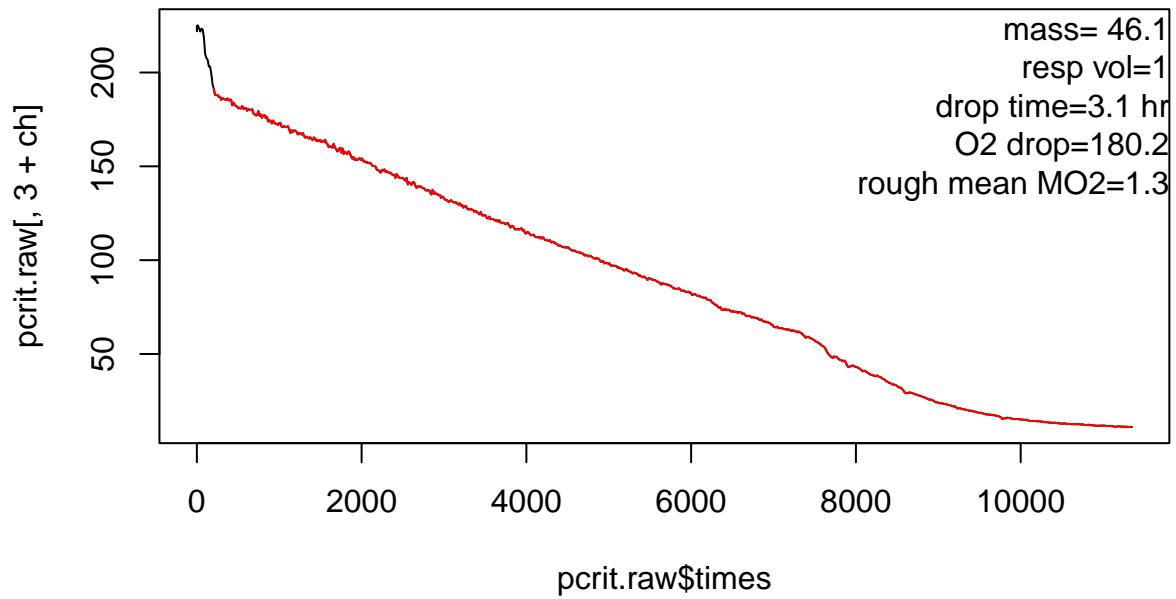
gr2MUUS1800-2pcritday7.8-3-21.txt



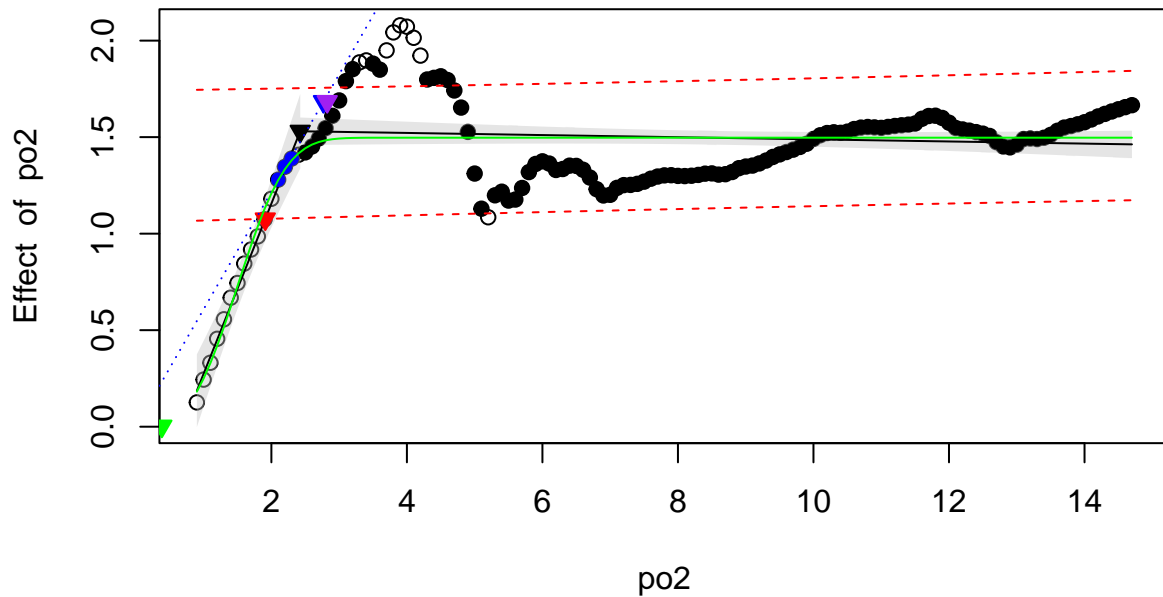
Alpha @ MR of 22.09 = 12.785
Breakpoint = 11.444
LLO @ MR of 22.09 = 14.387
NLR (Weibull with intercept) = 21
Sub-PI = 16



tbocto 1800 pcrit day 7 tank 10 blank ch 3 4 8-20-21-ch1.txt



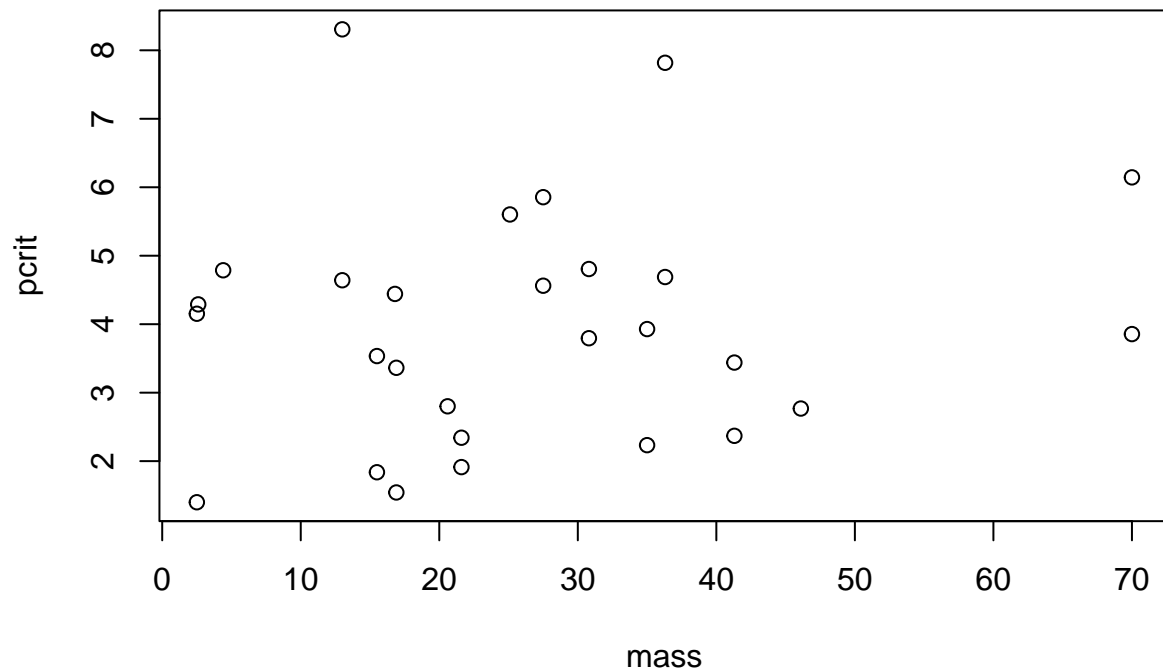
Alpha @ MR of 1.68 = 2.768
 Breakpoint = 2.426
 LLO @ MR of 1.68 = 2.824
 NLR (Weibull with intercept) = 0.384
 Sub-PI = 1.91



Next, I remove the measurements for which the data was excluded (runs did not reach oxygen level of 50 mmHgO₂). 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 (μ atm)	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 to be displayed in the RMarkdown.

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

Converting to eps for submission.

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

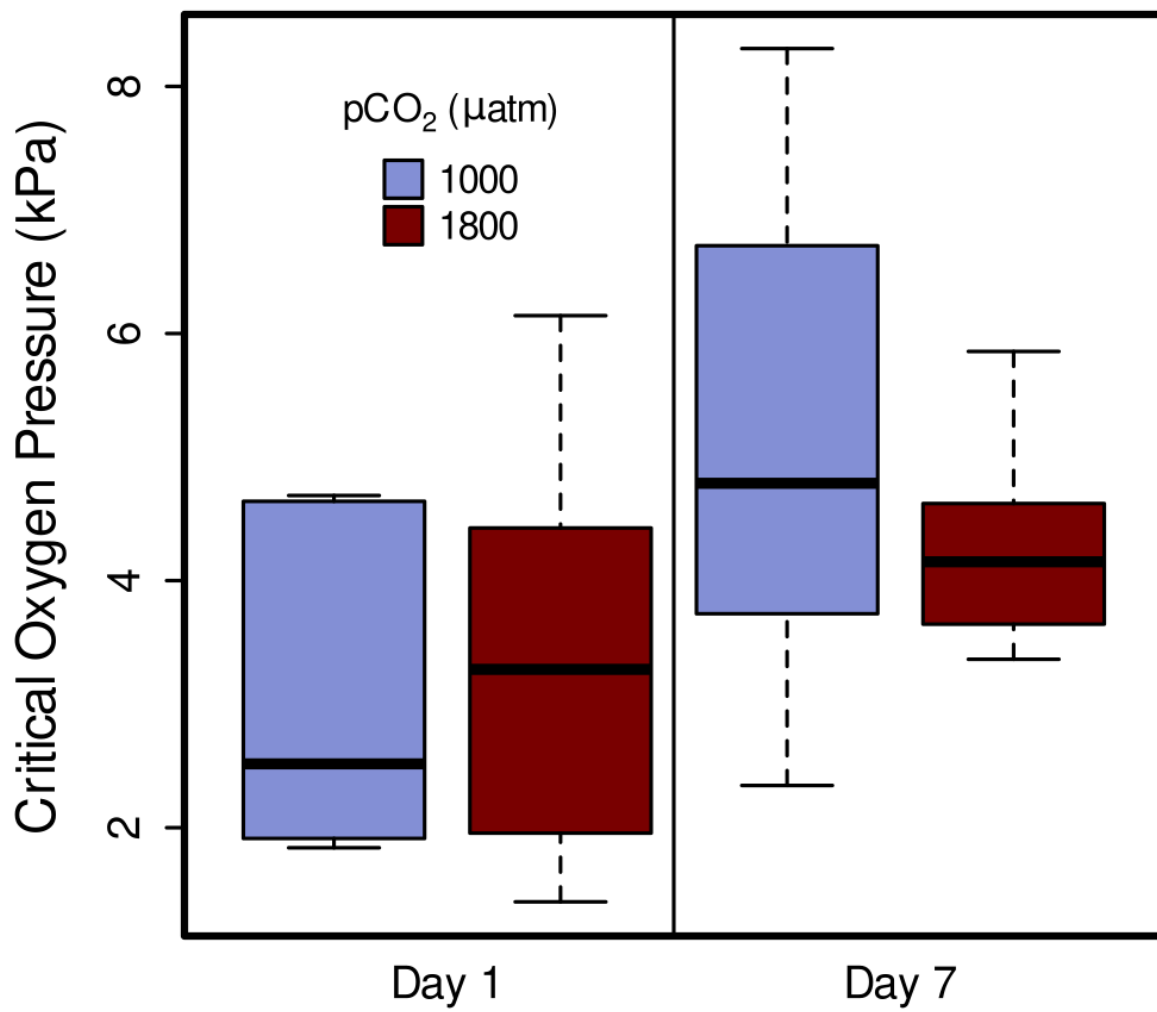


Figure 1: Critical oxygen pressure of *Muusoctopus leioderma* at differing pCO₂ treatments