

# Critical Oxygen Pressure ( $P_{\text{CRIT}}$ ) 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)
```

## 2 Sorting files for $P_{\text{CRIT}}$ 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 $P_{\text{CRIT}}$ from raw data

First I make a empty object where I can place calculated  $P_{\text{CRITS}}$

```
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  $P_{\text{CRIT}}$  from each data file. Here are a couple of important points on our calculations:

1. We are using the alpha  $P_{\text{CRIT}}$  method (Seibel et al, 2021) to calculate  $P_{\text{CRIT}}$  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  $P_{\text{CRIT}}$  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  $\text{MO}_2$  value from the oxyregulating portion of the curve (as defined by the broken-stick regression)”.
3. Any run in which  $\text{O}_2$  did not drop below 50 mmHg $\text{O}_2$  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  $P_{\text{CRIT}}$  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")

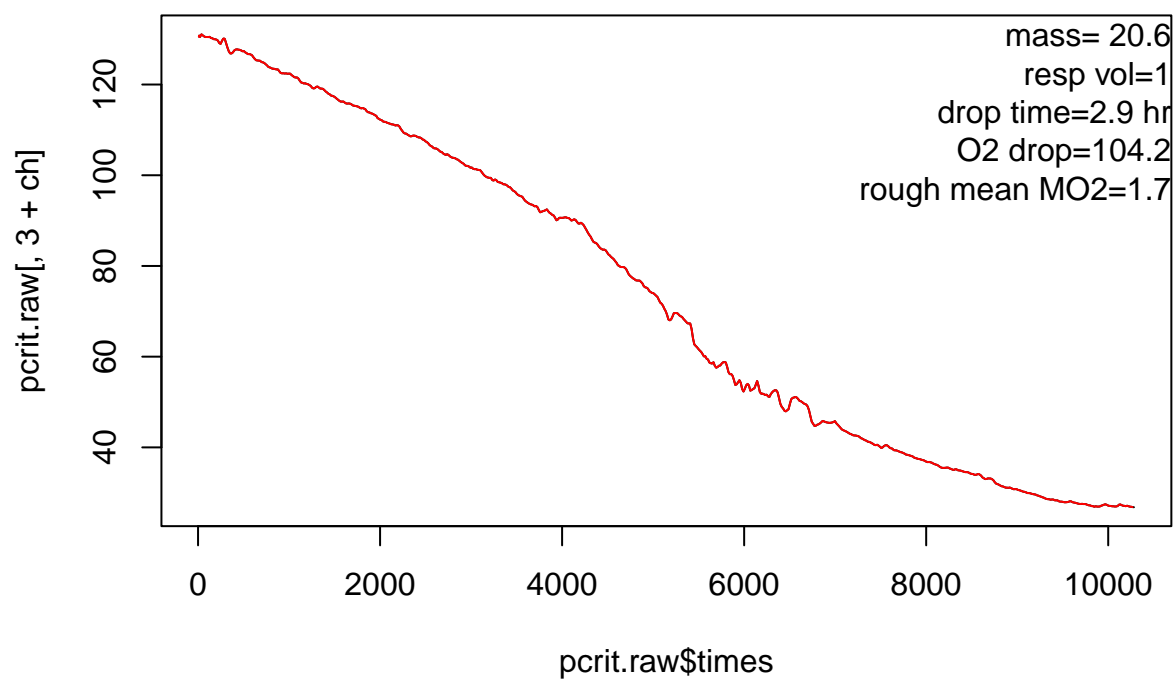
if(max(pcrit.bin$resp)>rmr){
  plot_pcrit(pcrit.bin$po2,pcrit.bin$resp,
    avg_top_n = 3,MR=rmr)
}else{
  plot_pcrit(pcrit.bin$po2,pcrit.bin$resp,
    avg_top_n = 3)
}

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
}

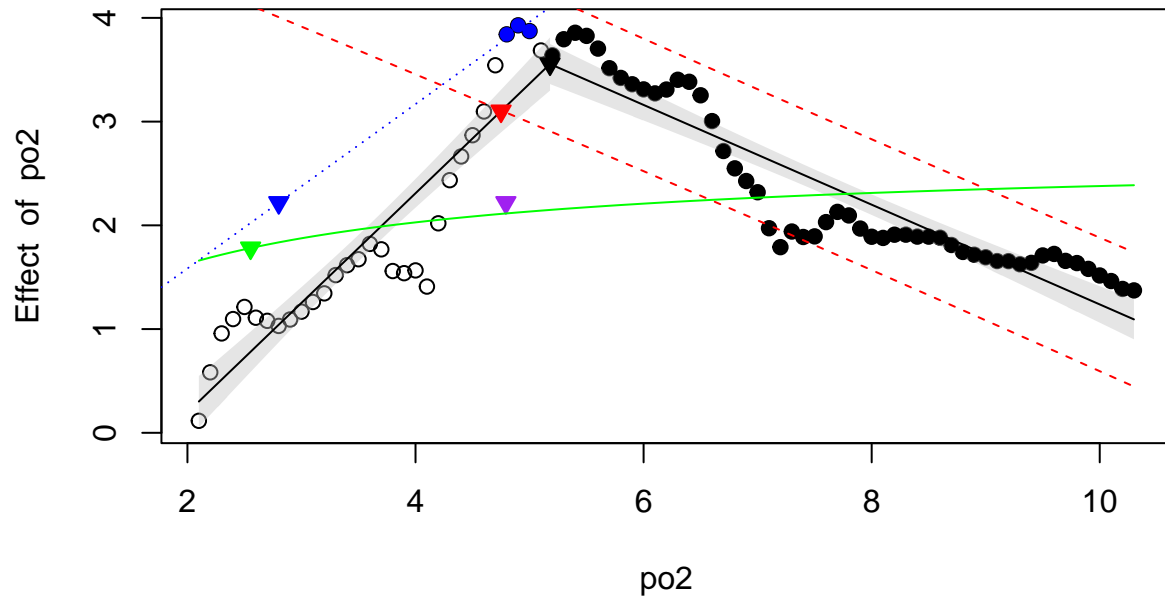
}

```

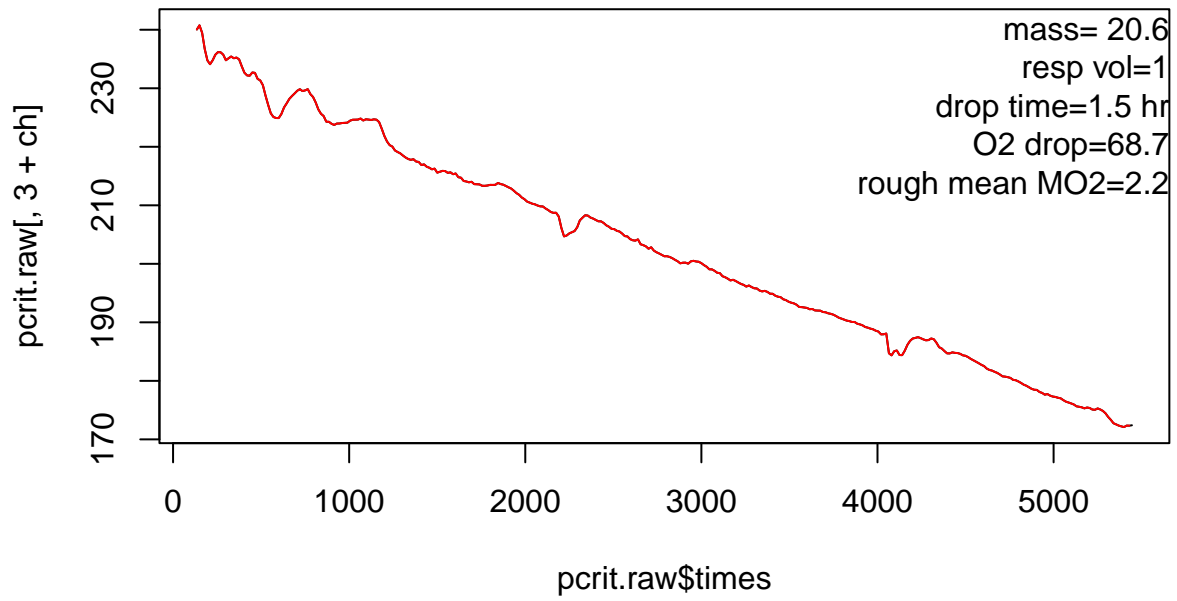
### Gr1 Muus 1000-2 pcrit 7-27-21 B.txt



**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





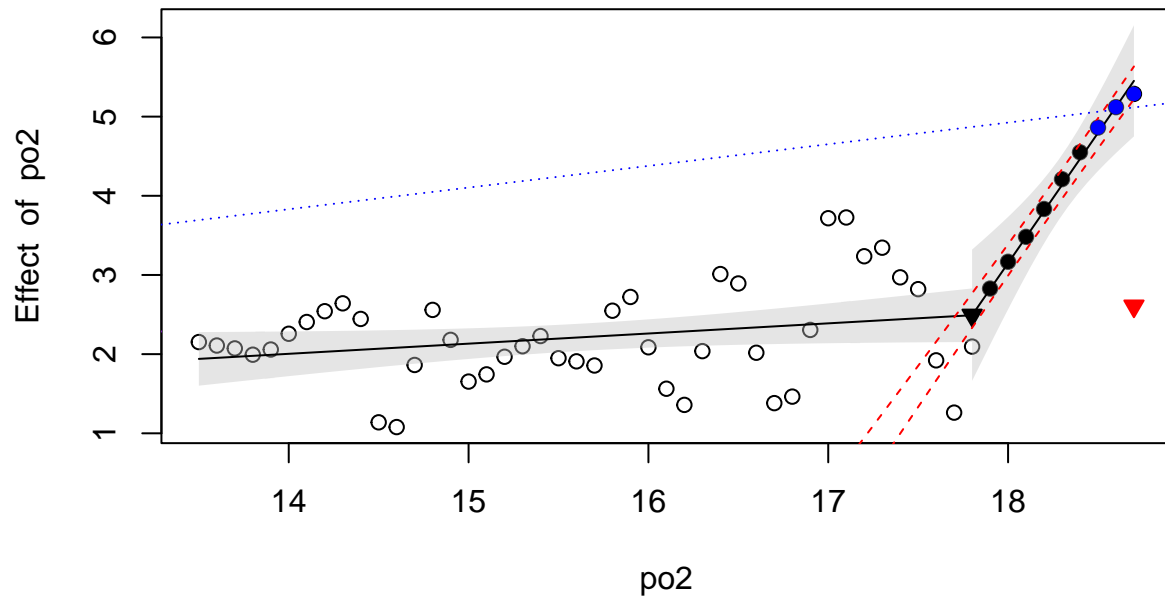
**Alpha @ MR of 2.22 = 8.109**

**Breakpoint = 17.8**

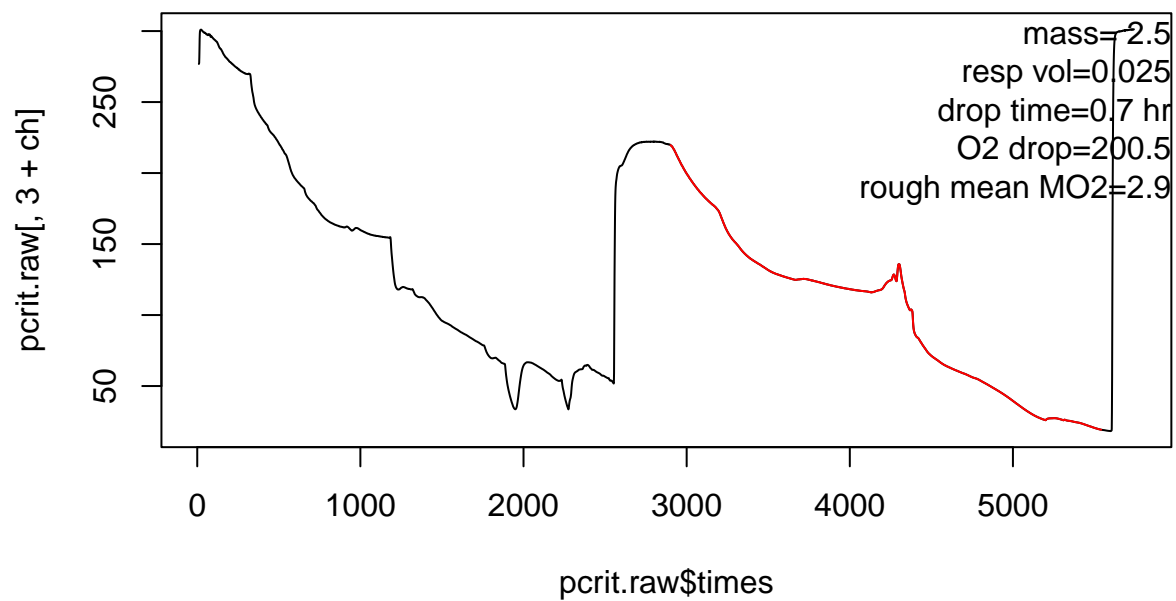
**LLO @ MR of 2.22 = 13.235**

**NLR () = NA**

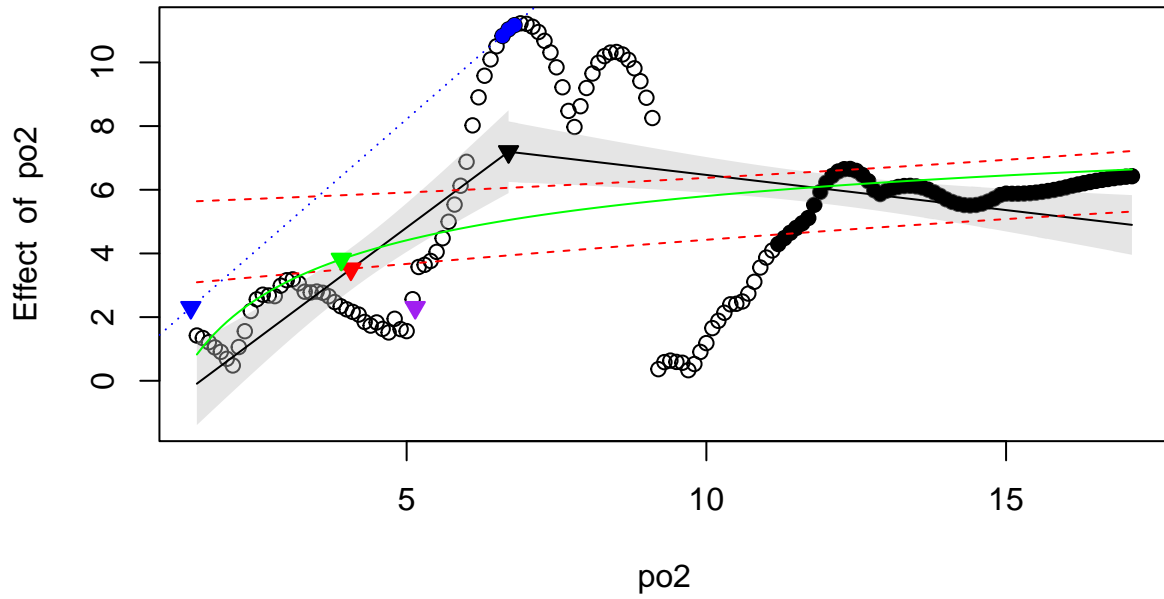
**Sub-PI = 18.7**



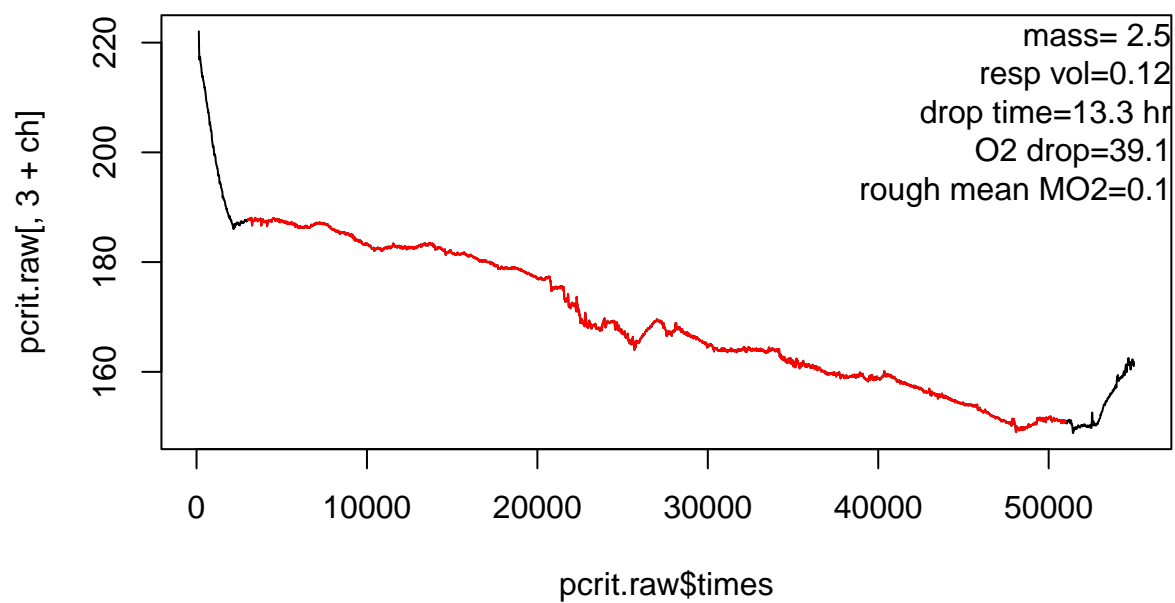
**Gr1 Muus 1800-2 pcrit 25 ml jar 7-29-21 ch2 blank.txt**



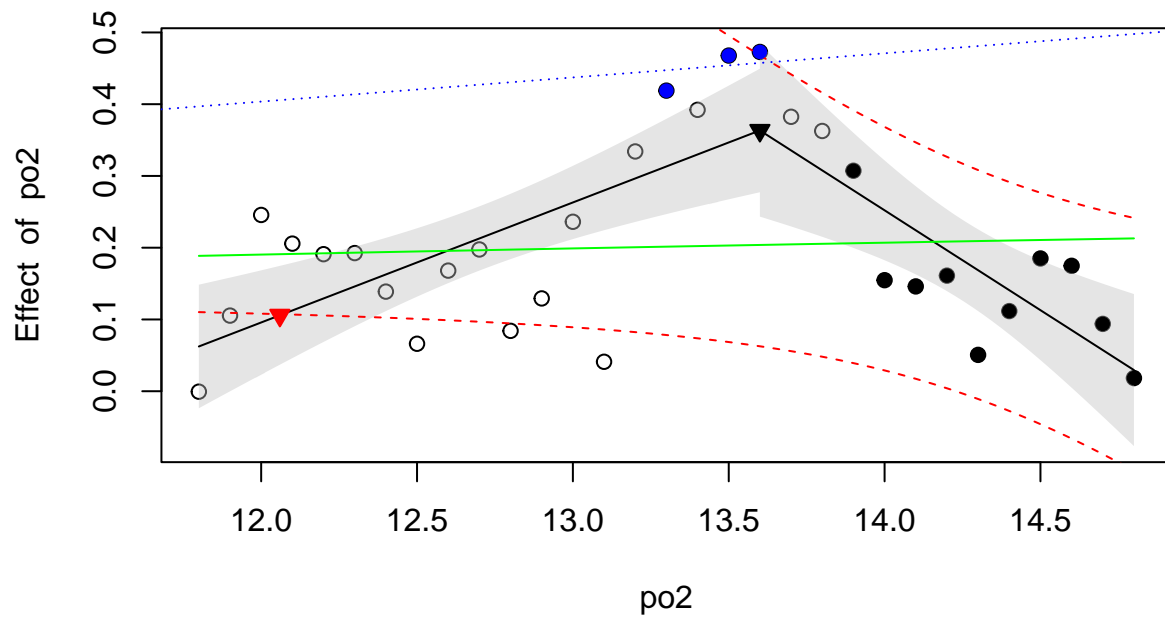
Alpha @ MR of 2.3 = 1.4  
Breakpoint = 6.7  
LLO @ MR of 2.3 = 5.143  
NLR (Pareto) = 3.91  
Sub-PI = 4.07



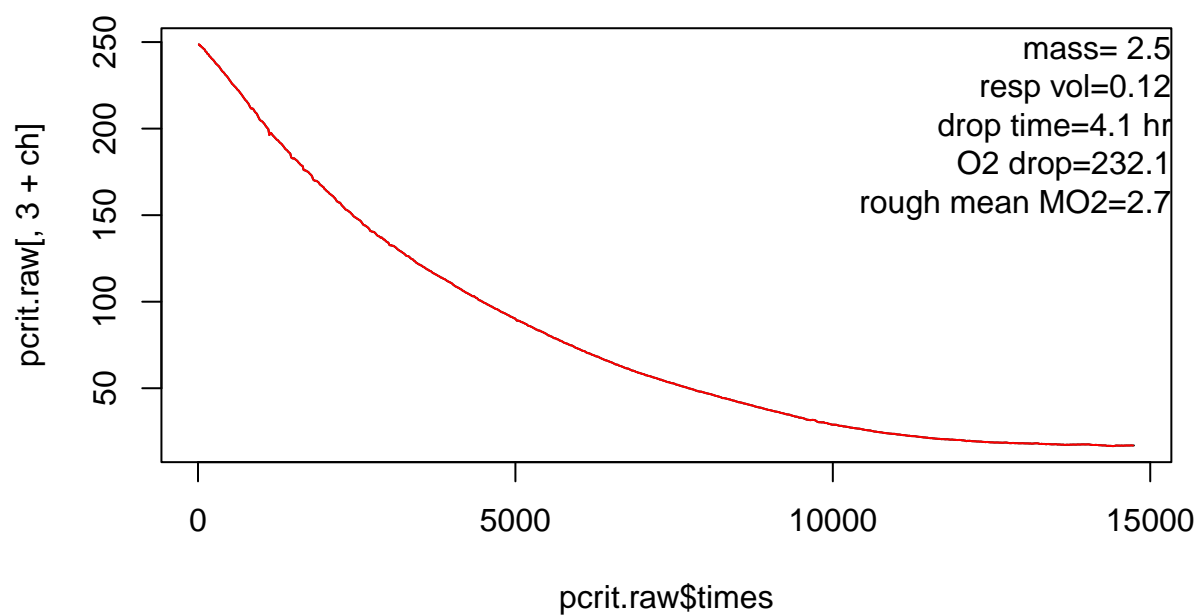
# Gr1 Muus 1800-2 pcrit 7-28-21.txt



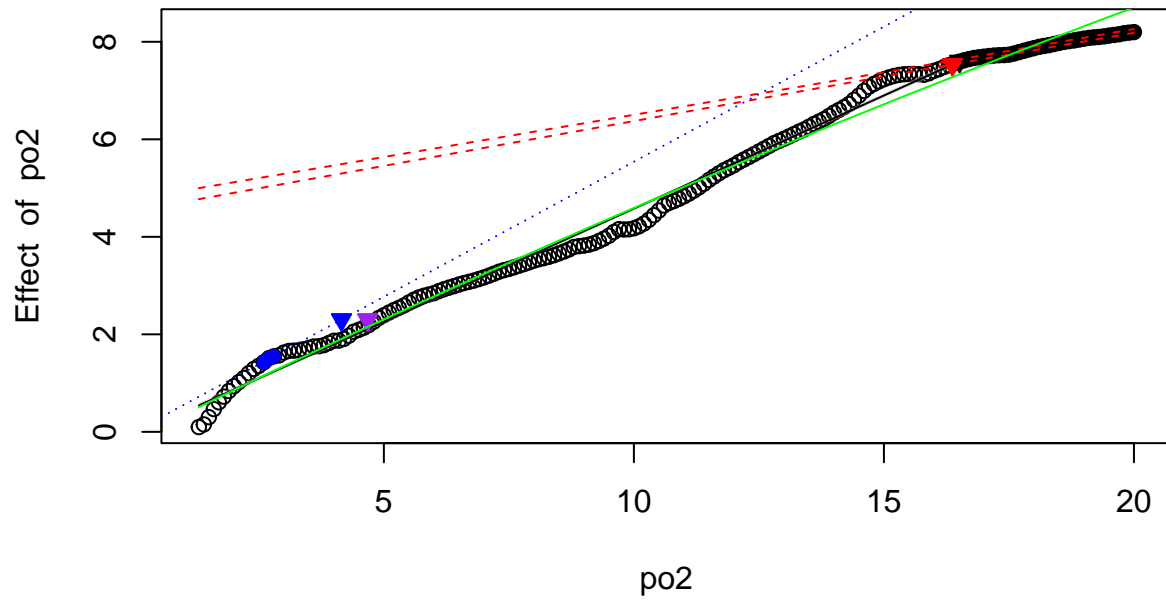
Alpha @ MR of 0.14 = 4.172  
 Breakpoint = 13.6  
 LLO @ MR of NA = NA  
 NLR (Michaelis-Menten) = 0.829  
 Sub-PI = 12.06



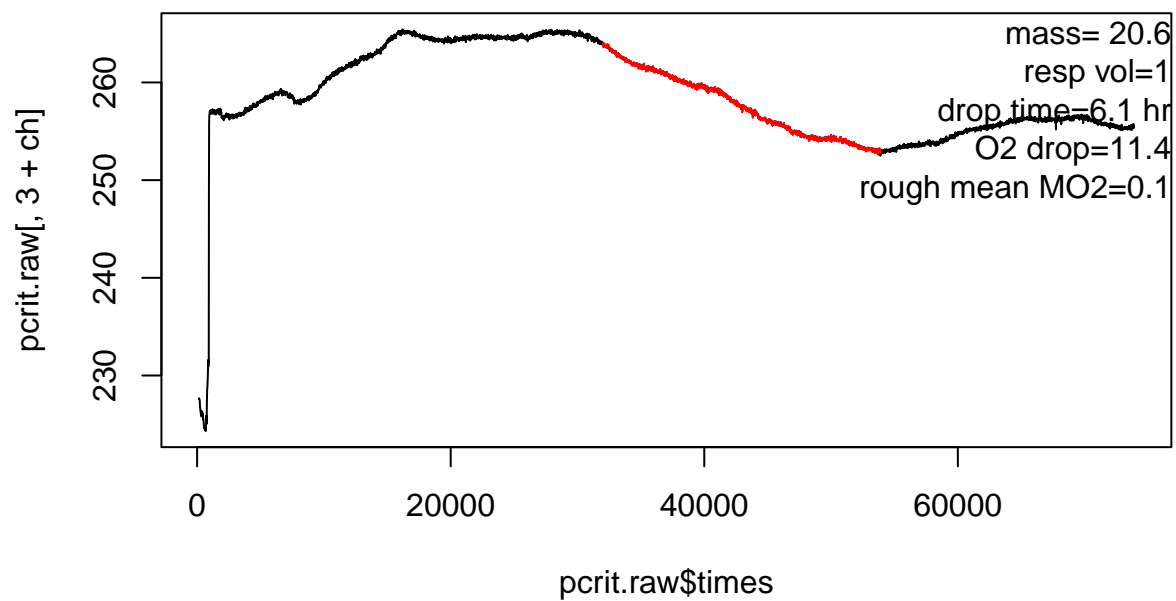
# GR1 Muus 1800-2 pcrit day7 8-3-21.txt



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





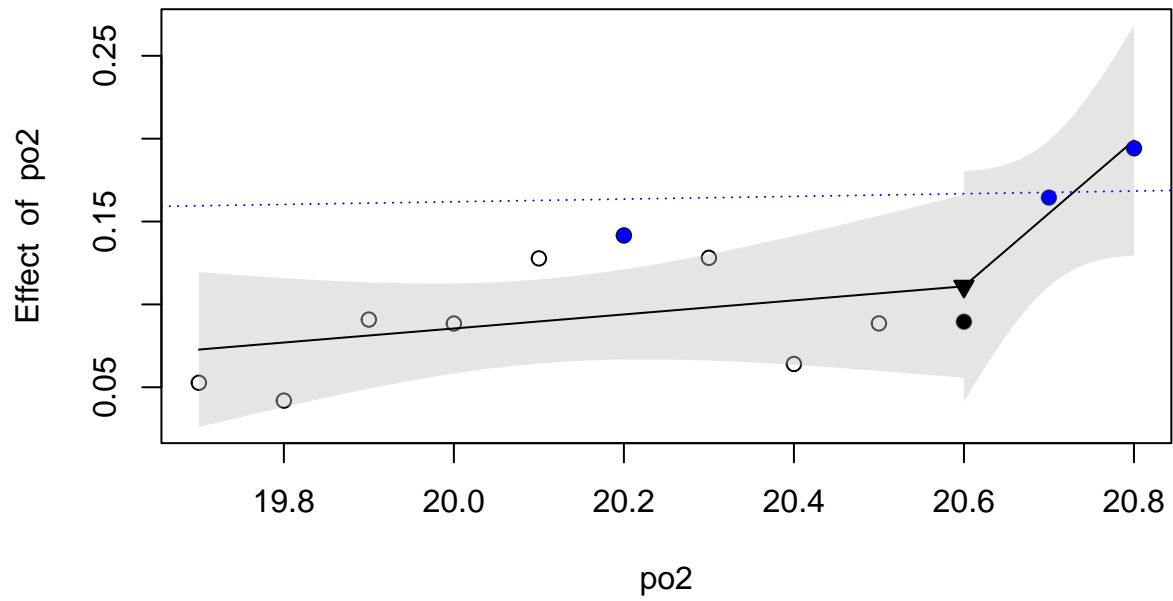
Alpha @ MR of 0.15 = 18.451

Breakpoint = 20.6

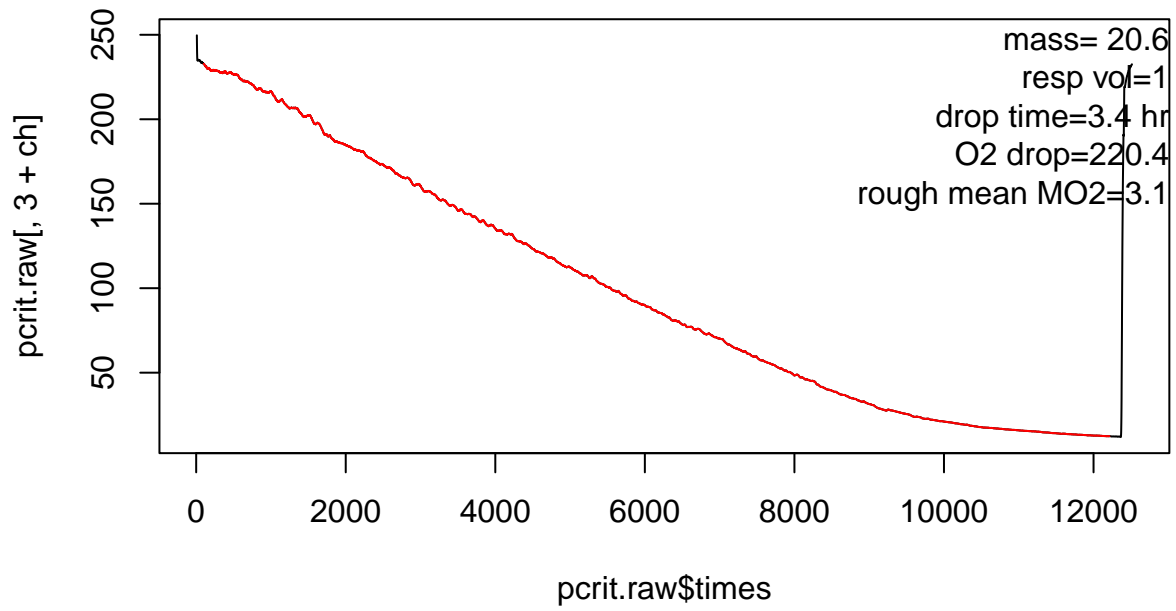
LLO @ MR of NA = NA

NLR () = NA

Sub-PI =



# GR1 Muus1000 pcrit 7-21-21.txt



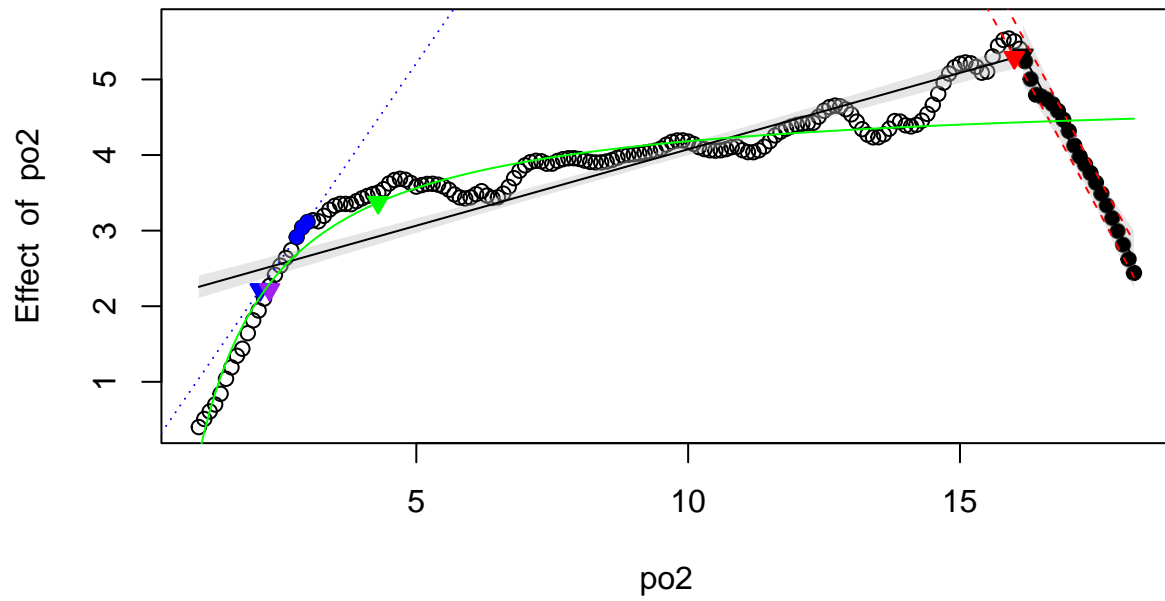
**Alpha @ MR of 2.22 = 2.128**

**Breakpoint = 16.161**

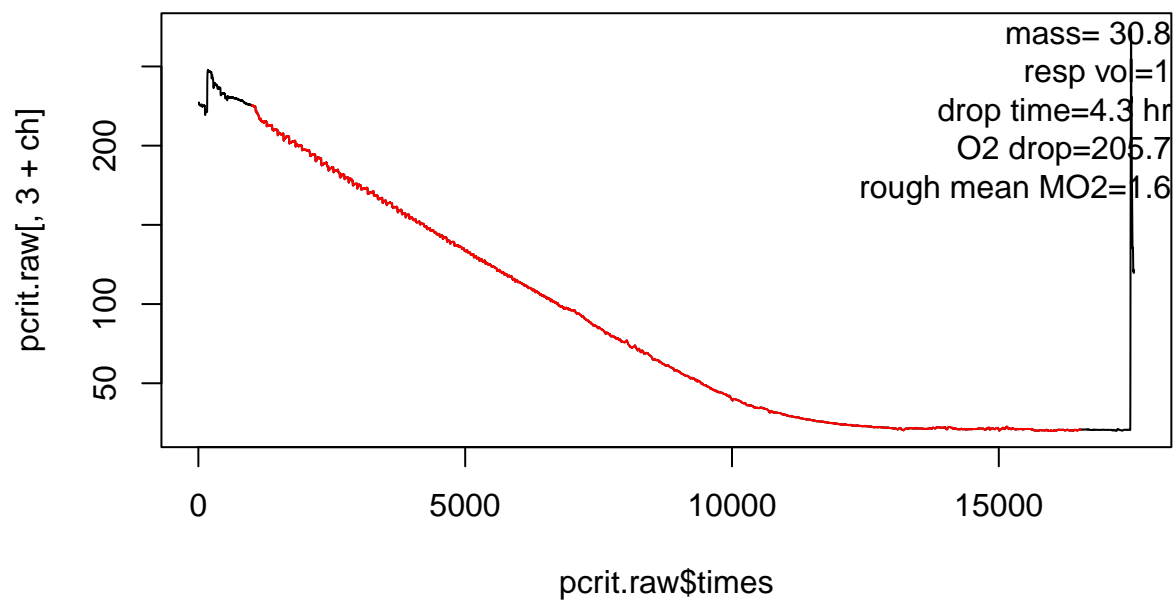
**LLO @ MR of 2.22 = 2.3**

**NLR (Hyperbola) = 4.298**

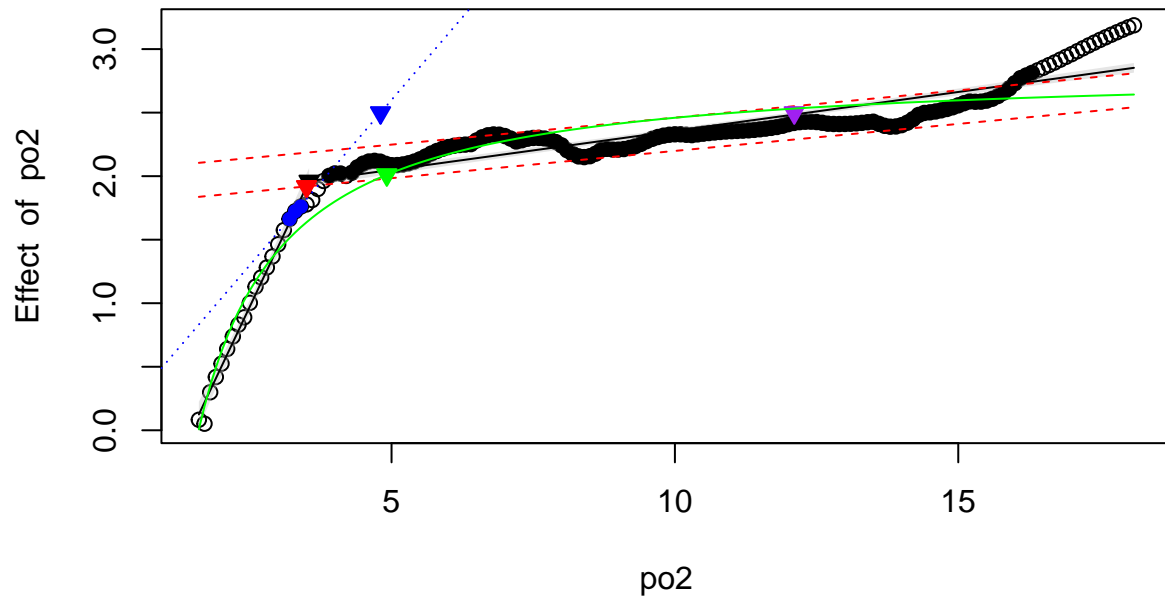
**Sub-PI = 16**



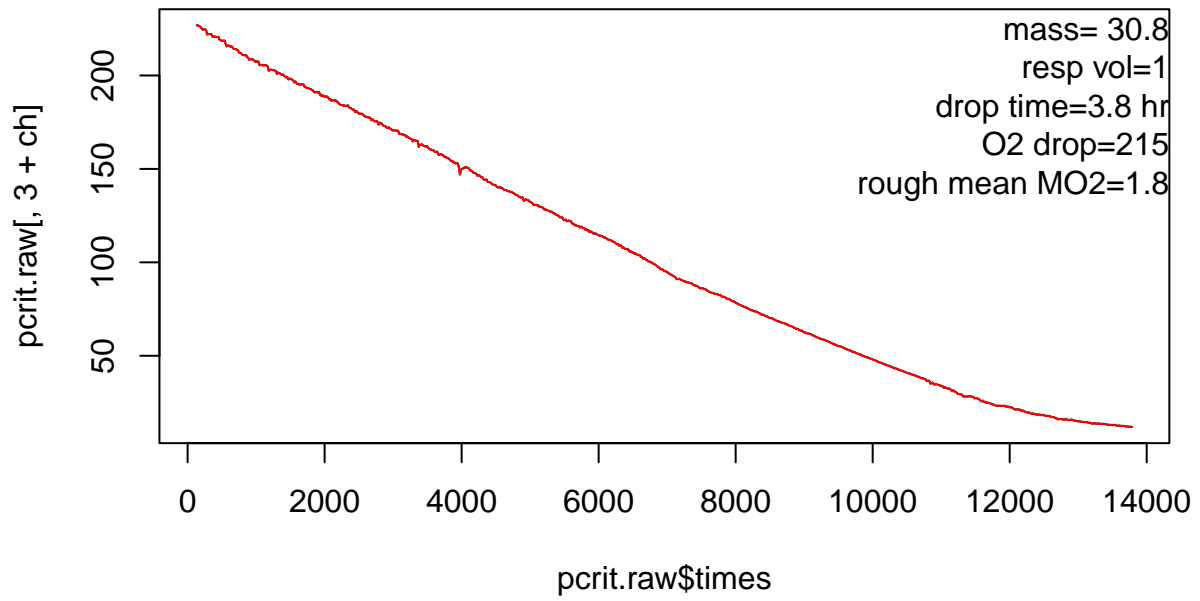
# GR1 Muus1800 7day-pcrit 7-20-21.txt



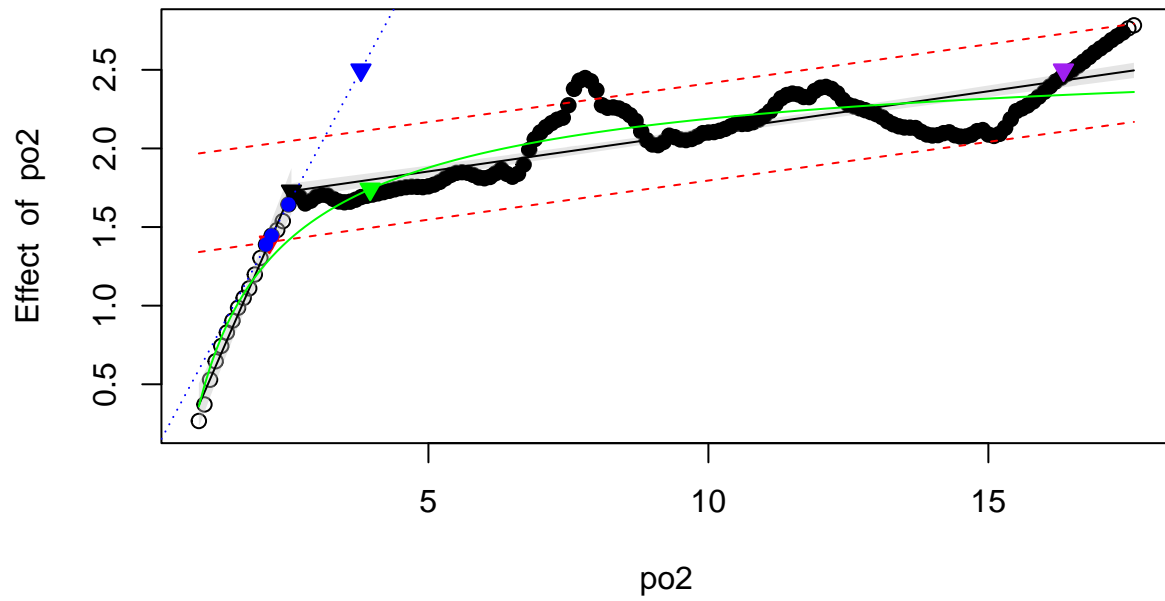
**Alpha @ MR of 2.5 = 4.806**  
**Breakpoint = 3.545**  
**LLO @ MR of 2.5 = 12.106**  
**NLR (Pareto) = 4.917**  
**Sub-PI = 3.5**



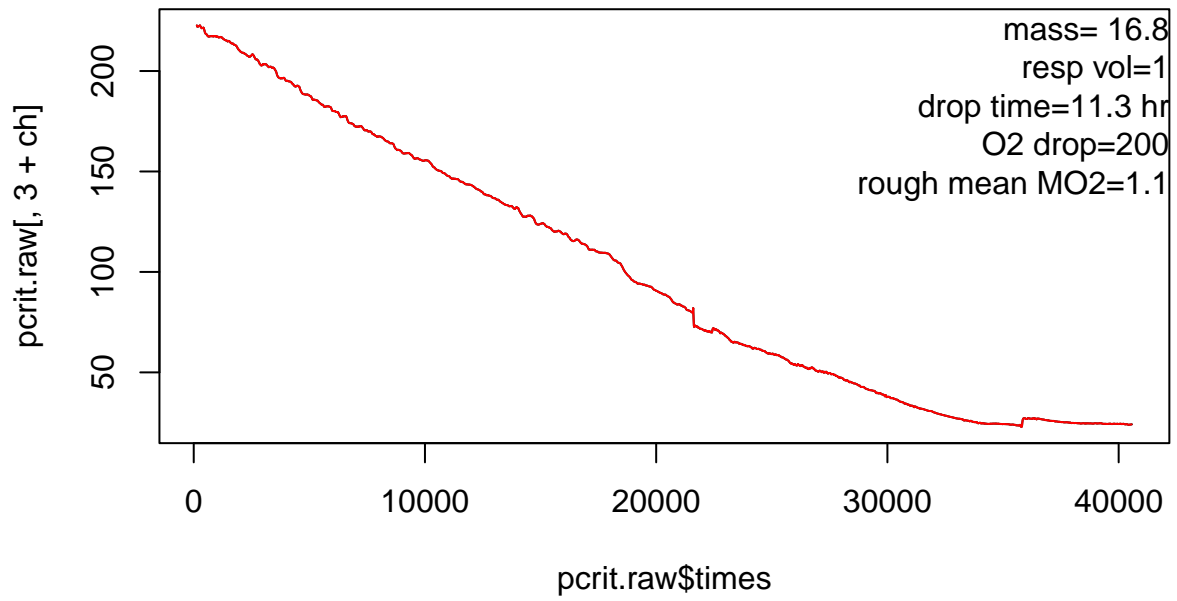
# GR1 Muus1800 pcrit 7-13-21.txt



**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





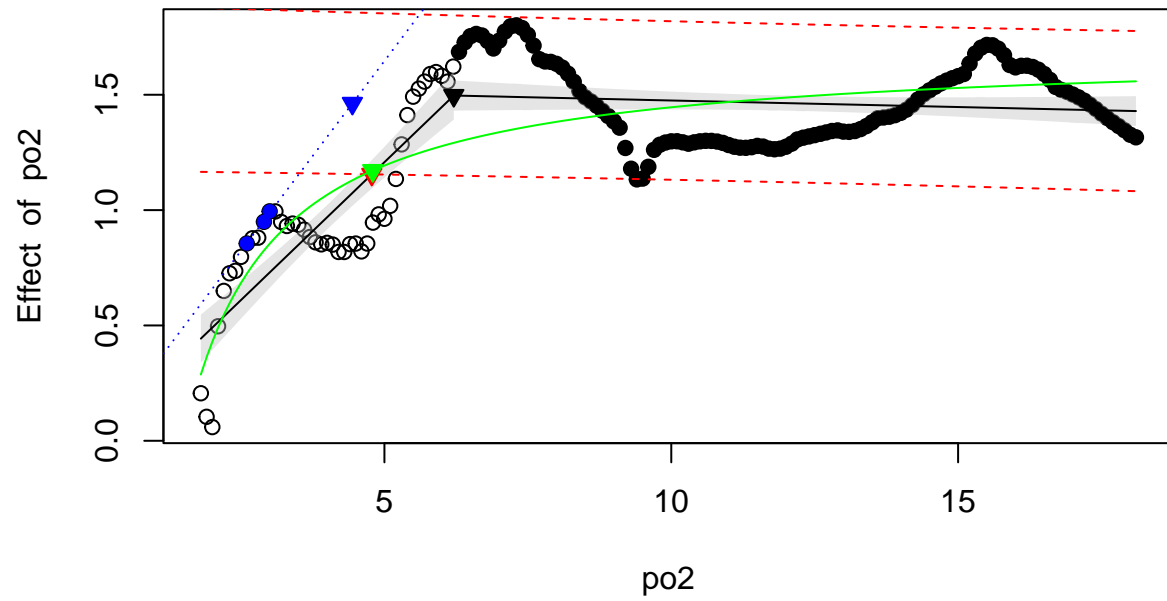
**Alpha @ MR of 1.46 = 4.442**

**Breakpoint = 6.209**

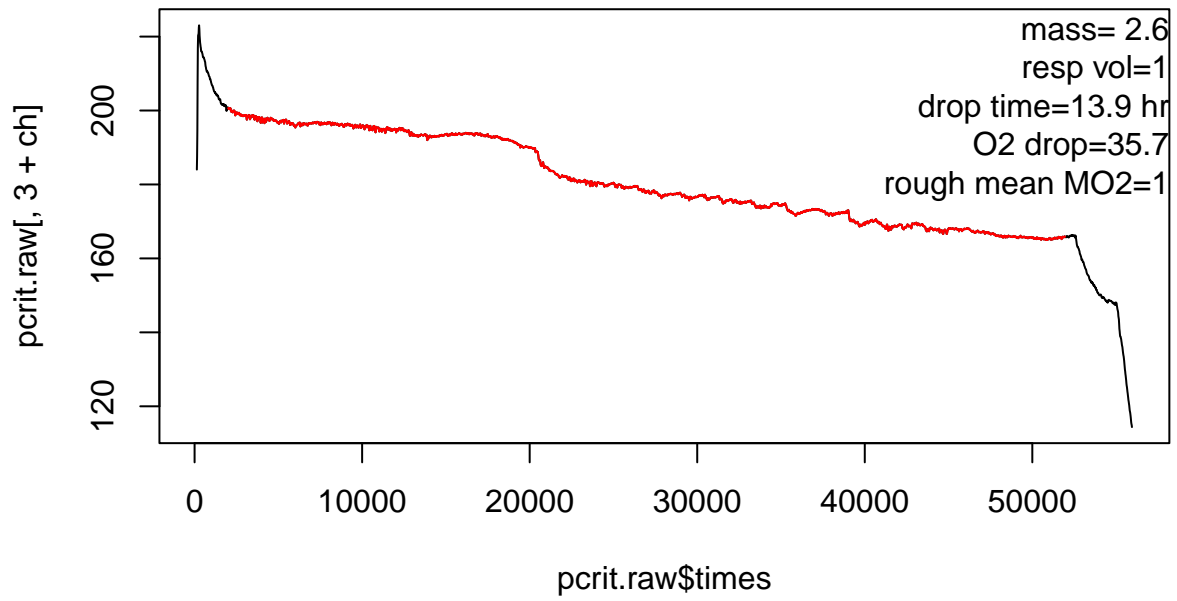
**LLO @ MR of NA = NA**

**NLR (Pareto) = 4.796**

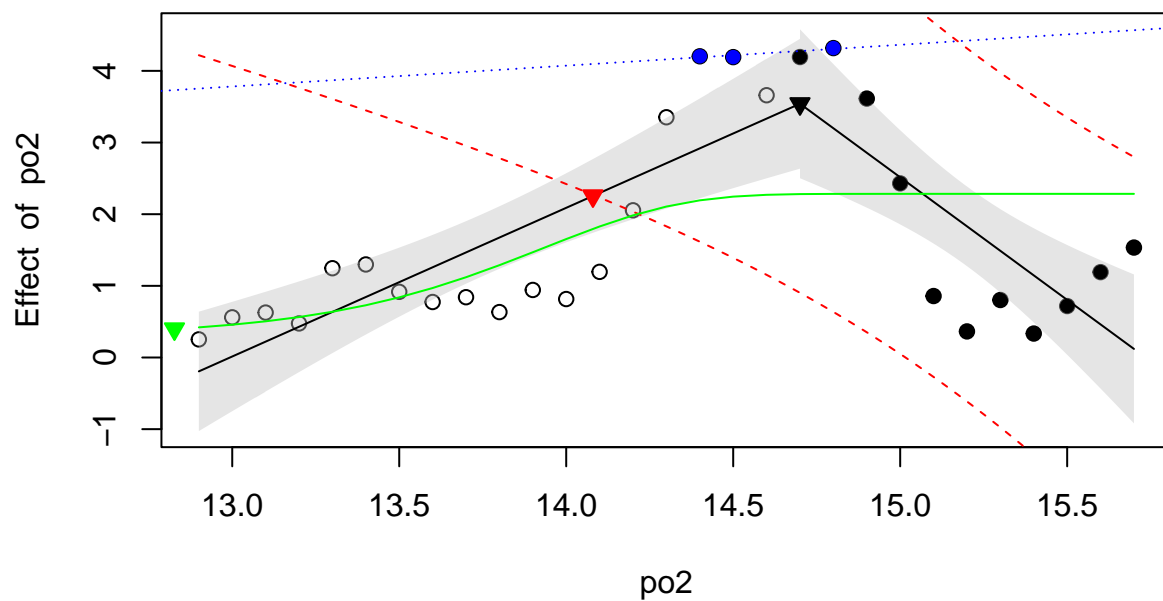
**Sub-PI = 4.78**



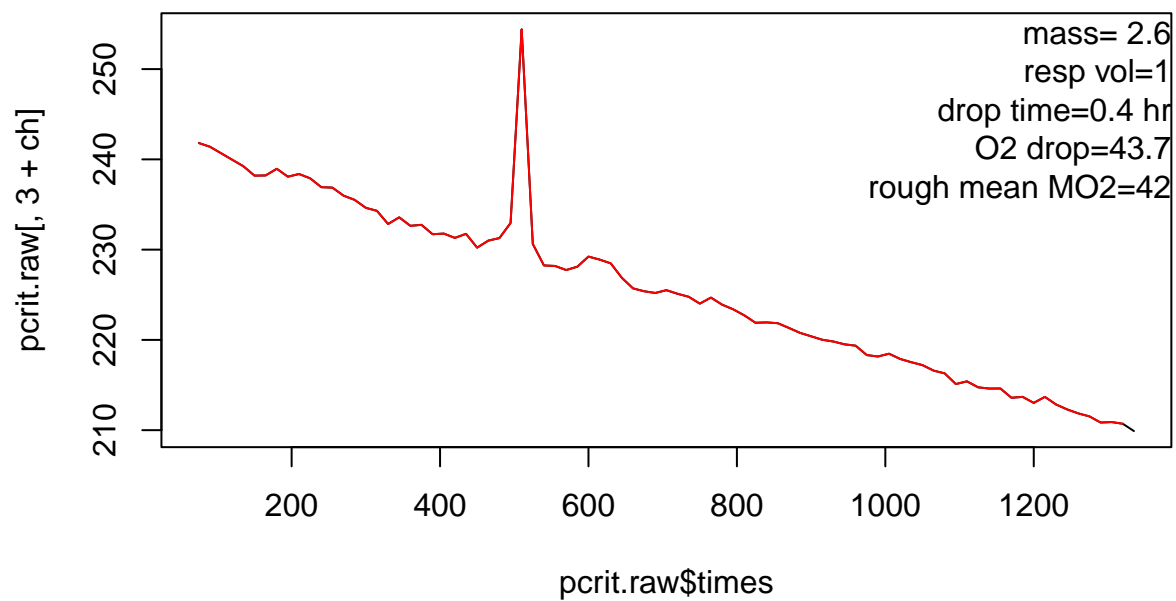
# gr2muus1800-2 pcrit 7-28-21.txt



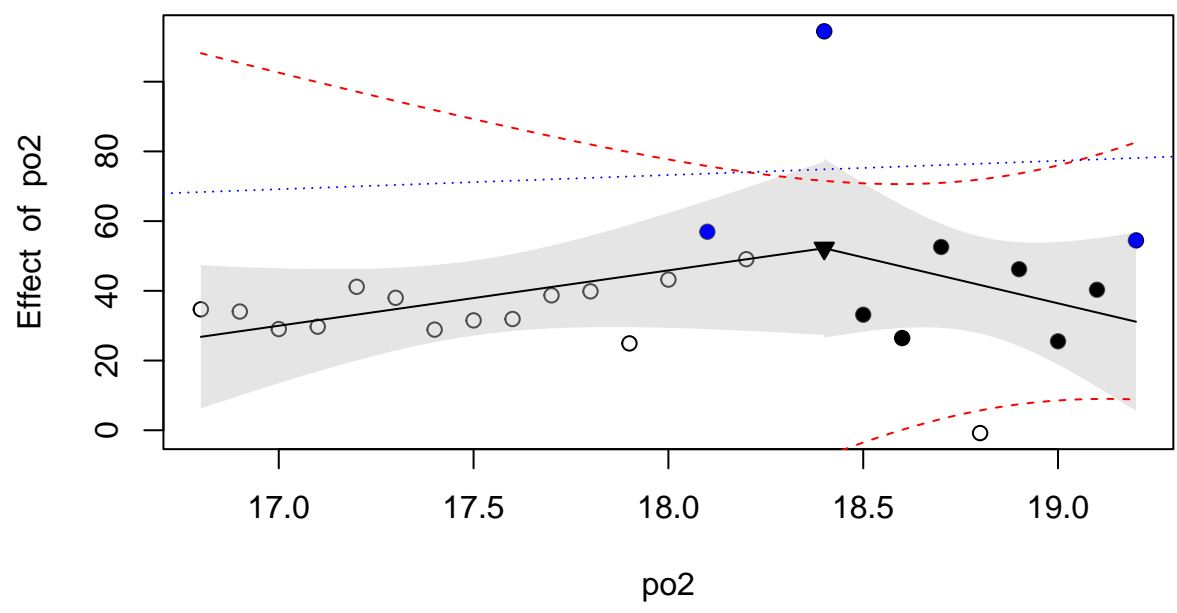
Alpha @ MR of 1.85 = 6.361  
 Breakpoint = 14.7  
 LLO @ MR of NA = NA  
 NLR (Weibull with intercept) = 12.827  
 Sub-PI = 14.08



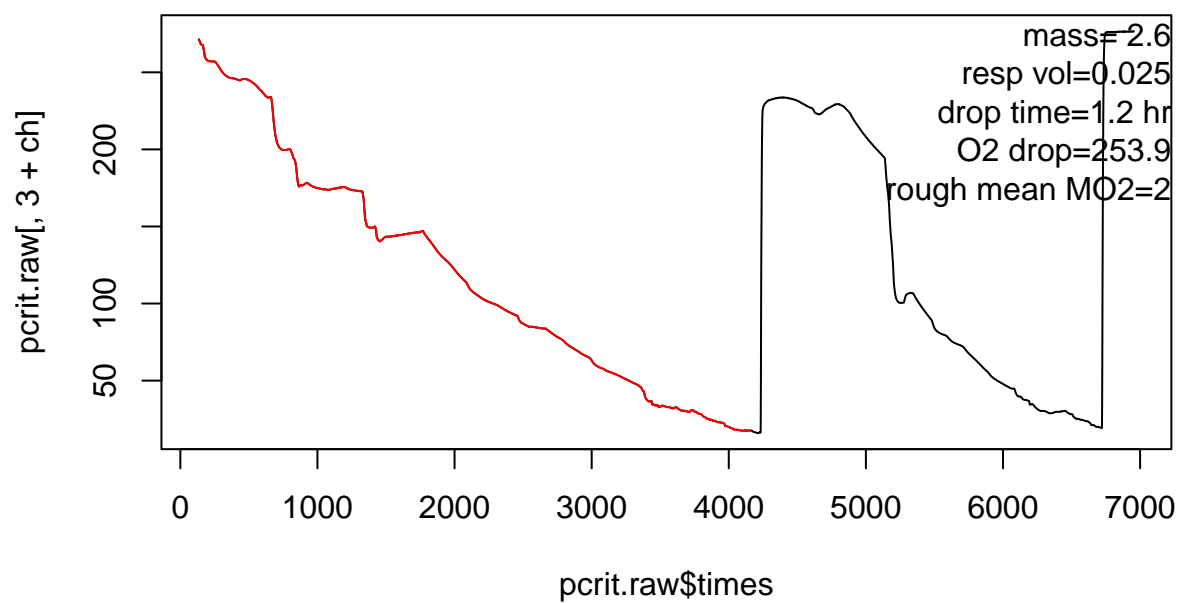
**gr2muus1800-2 pcrit day7 8-3-21.txt**



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



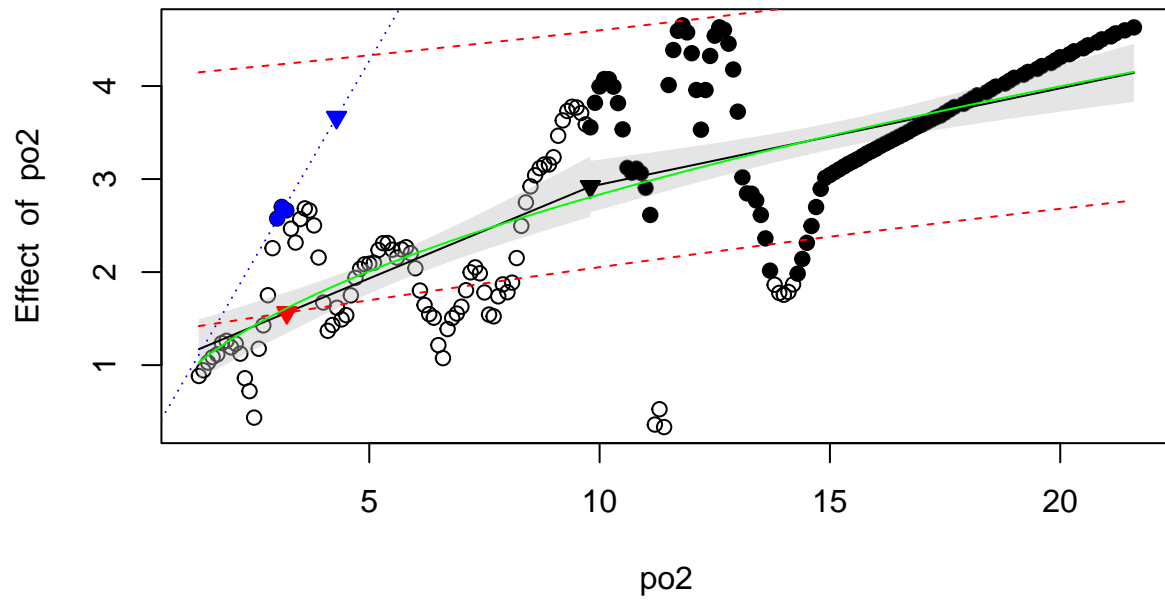
**Alpha @ MR of 3.66 = 4.289**

**Breakpoint = 9.8**

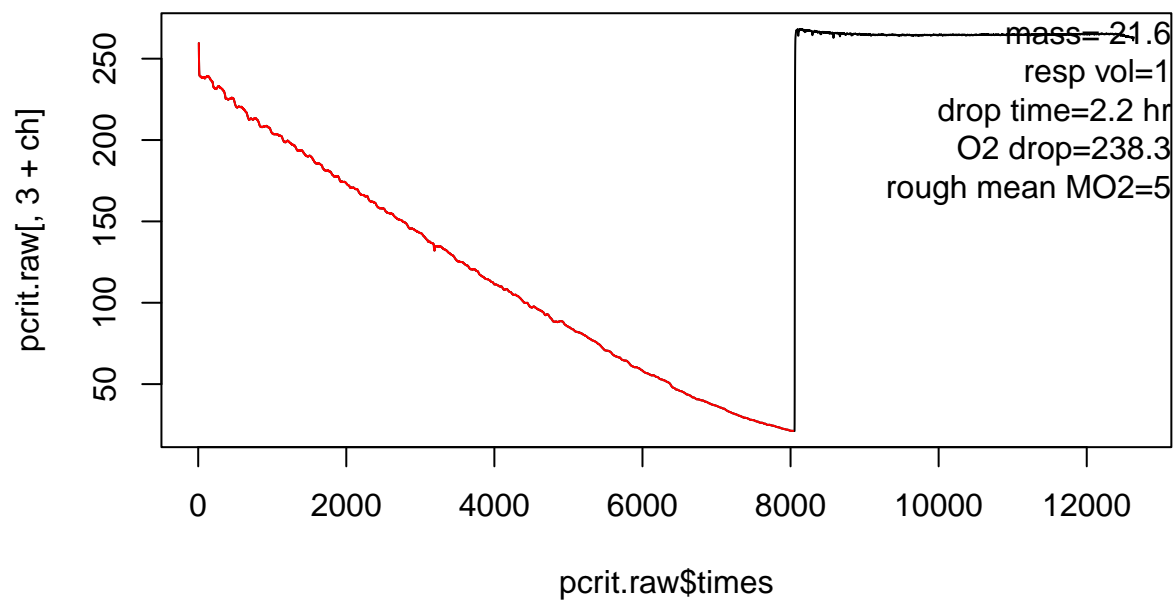
**LLO @ MR of NA = NA**

**NLR (Power) = 40.873**

**Sub-PI = 3.21**



### Gr3 Muus 1000 pcrit 7-21-21.txt





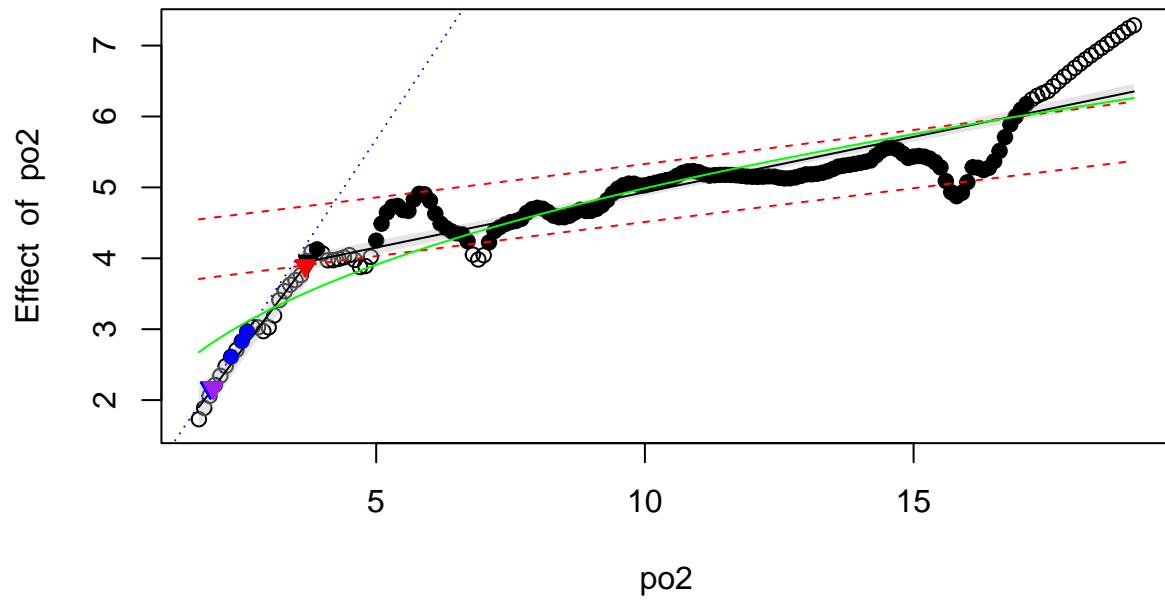
**Alpha @ MR of 2.17 = 1.913**

**Breakpoint = 3.74**

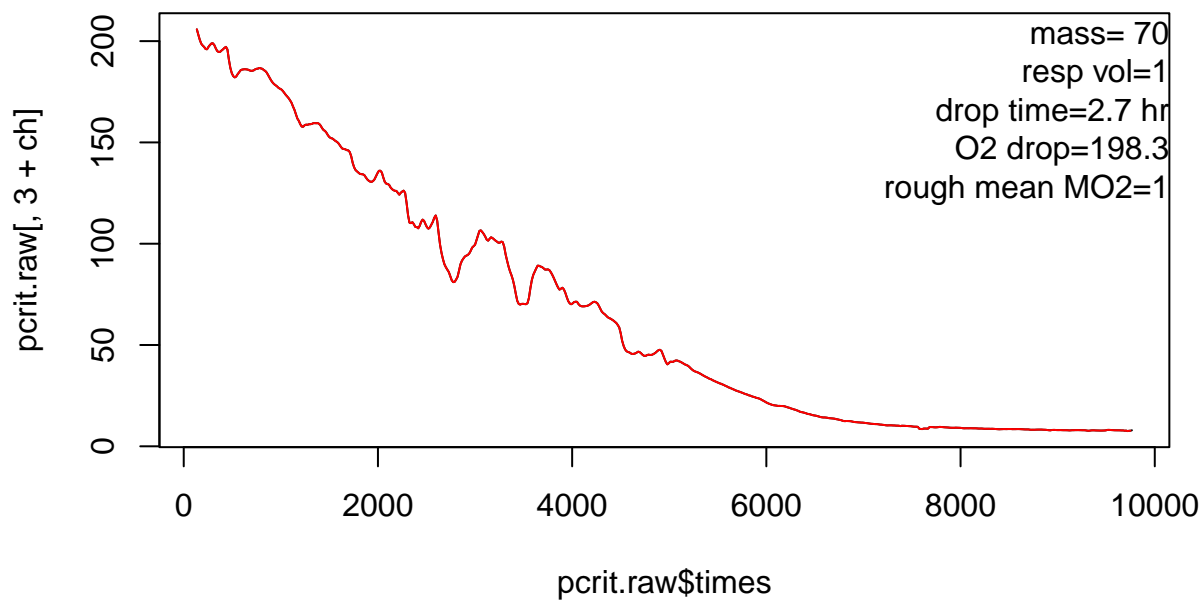
**LLO @ MR of 2.17 = 1.97**

**NLR (Power) = 67.77**

**Sub-PI = 3.68**



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



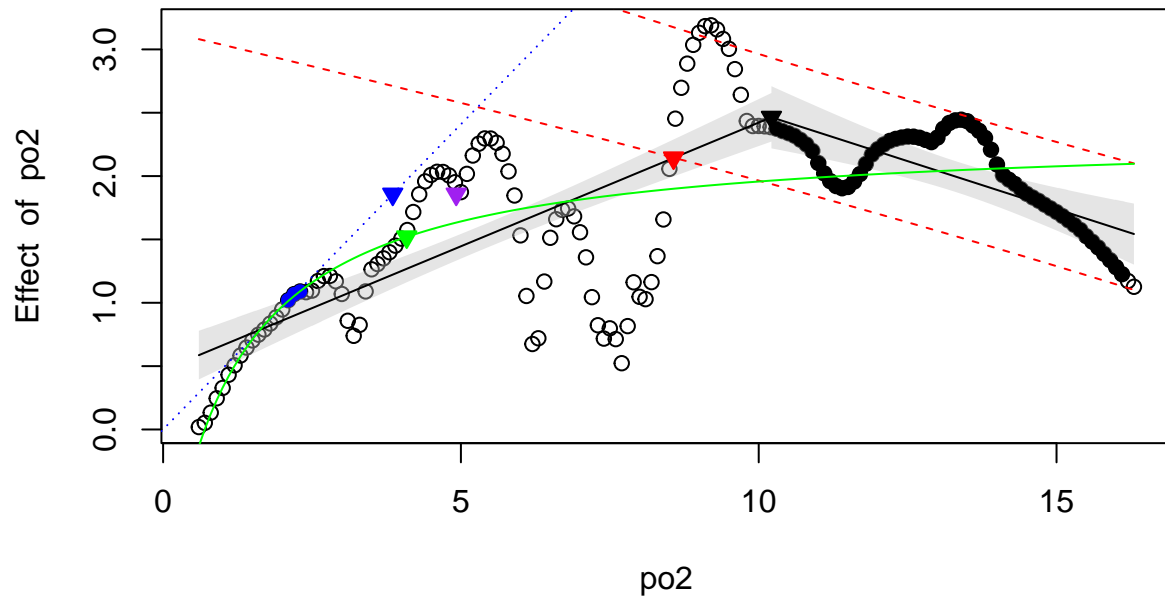
**Alpha @ MR of 1.86 = 3.855**

**Breakpoint = 10.214**

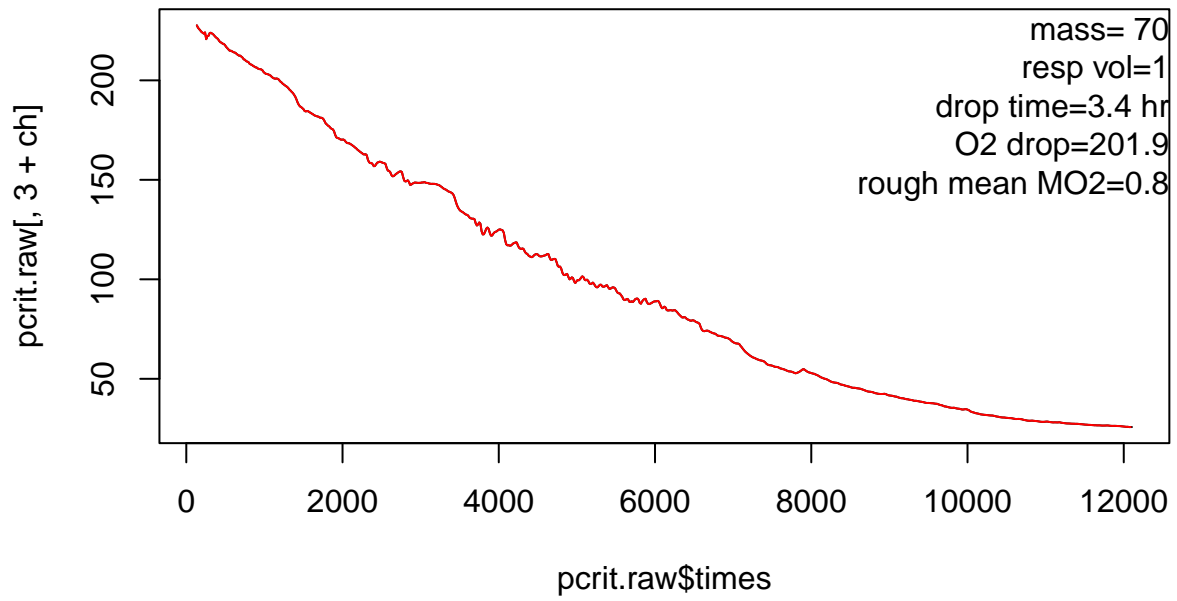
**LLO @ MR of 1.86 = 4.921**

**NLR (Hyperbola) = 4.09**

**Sub-PI = 8.57**



### gr3 muus 1800 pcrit 7-13-21.txt



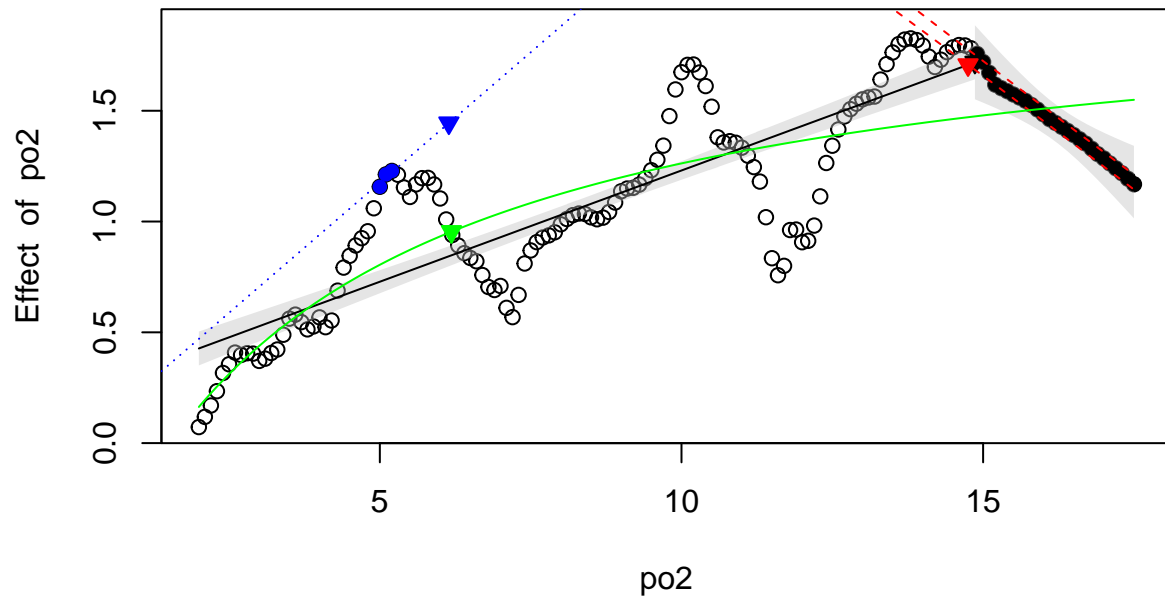
**Alpha @ MR of 1.44 = 6.145**

**Breakpoint = 14.864**

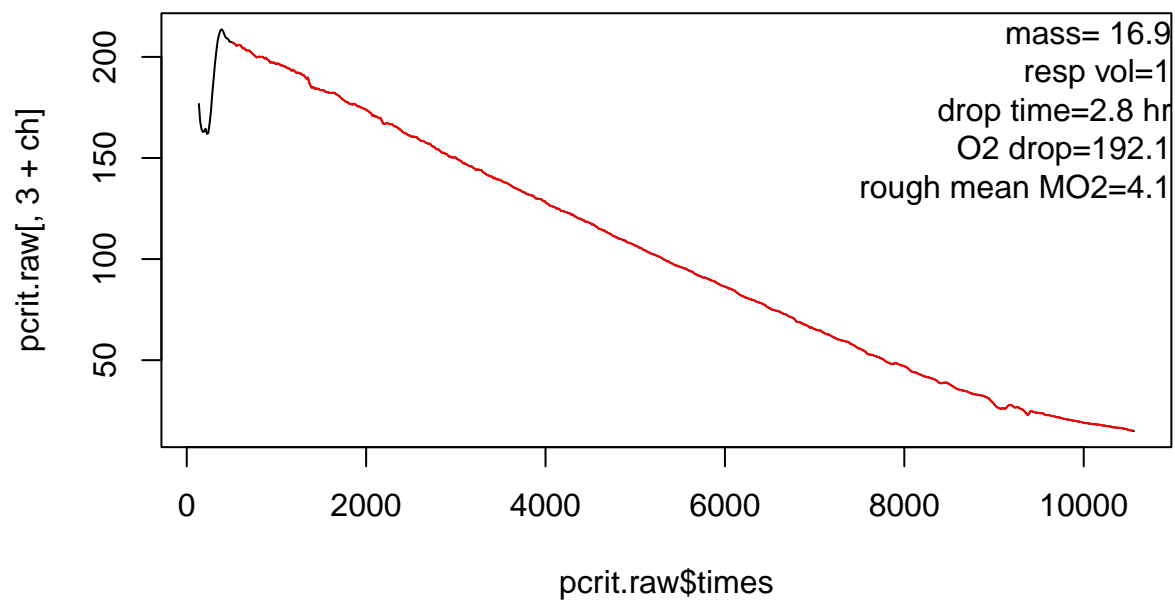
**LLO @ MR of NA = NA**

**NLR (Hyperbola) = 6.188**

**Sub-PI = 14.75**



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



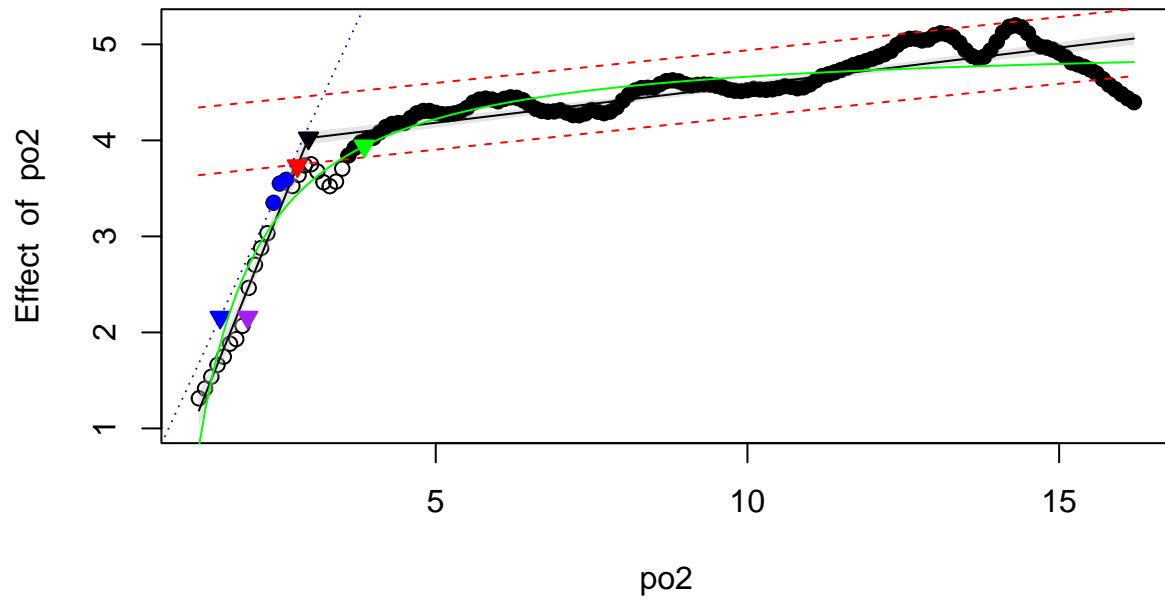
**Alpha @ MR of 2.16 = 1.542**

**Breakpoint = 2.959**

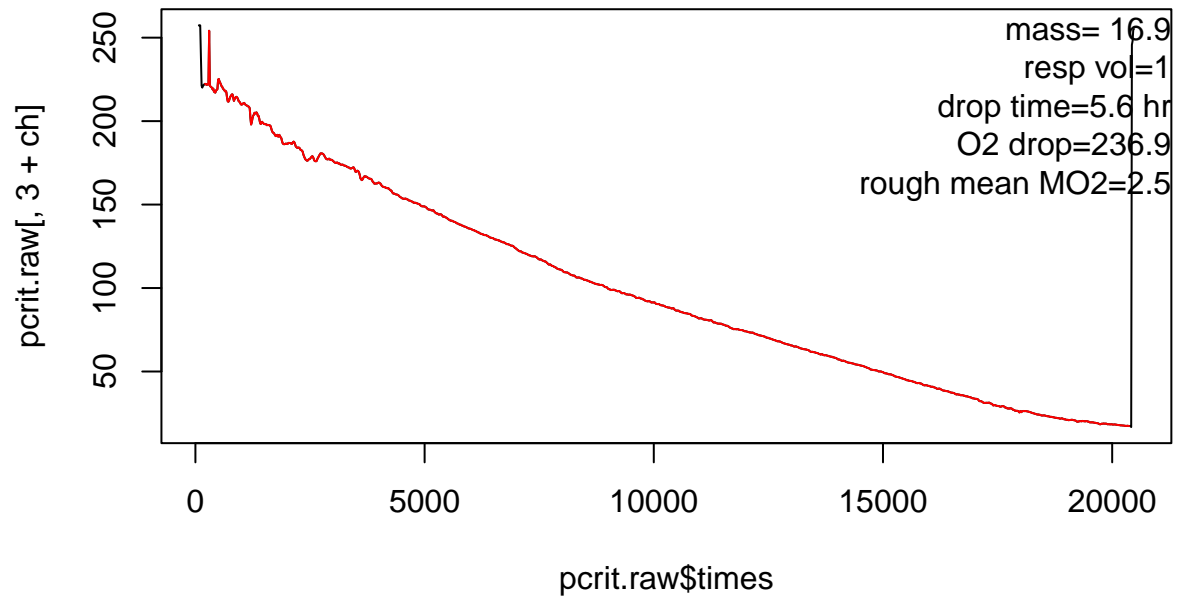
**LLO @ MR of 2.16 = 1.983**

**NLR (Pareto) = 3.857**

**Sub-PI = 2.78**

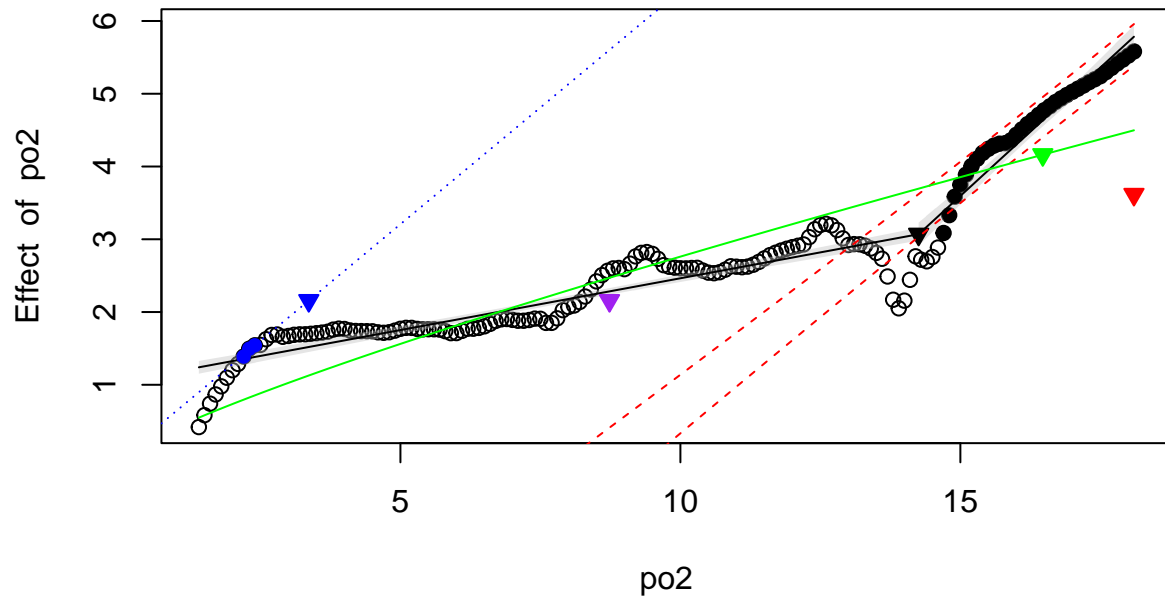


### Gr3 Muus 1800-2 pcrit 08-03-21.txt

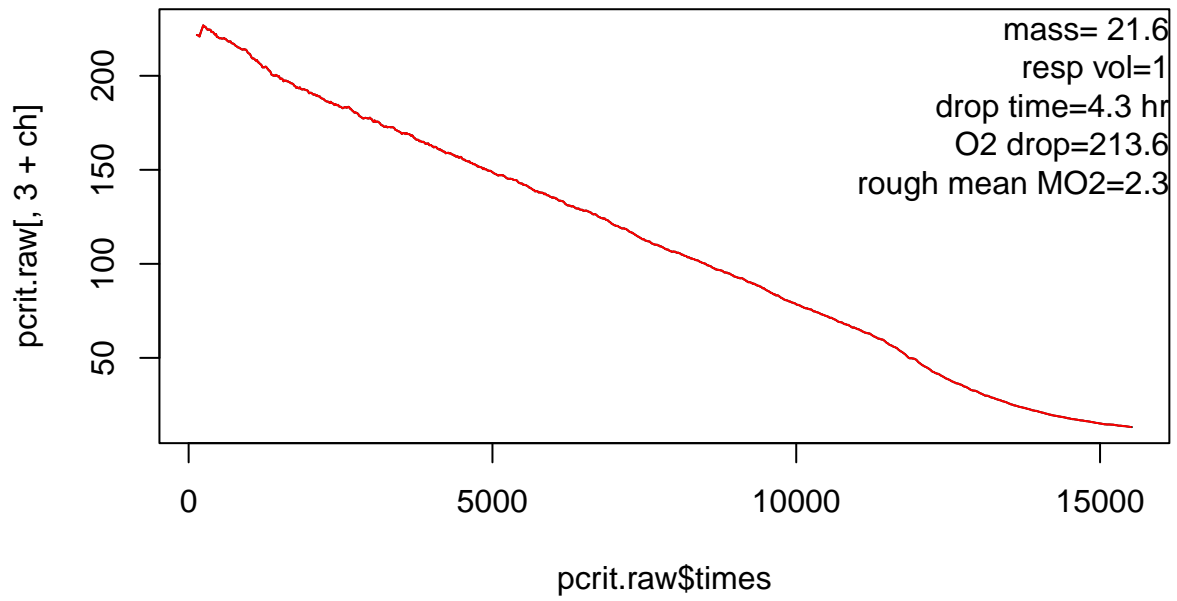




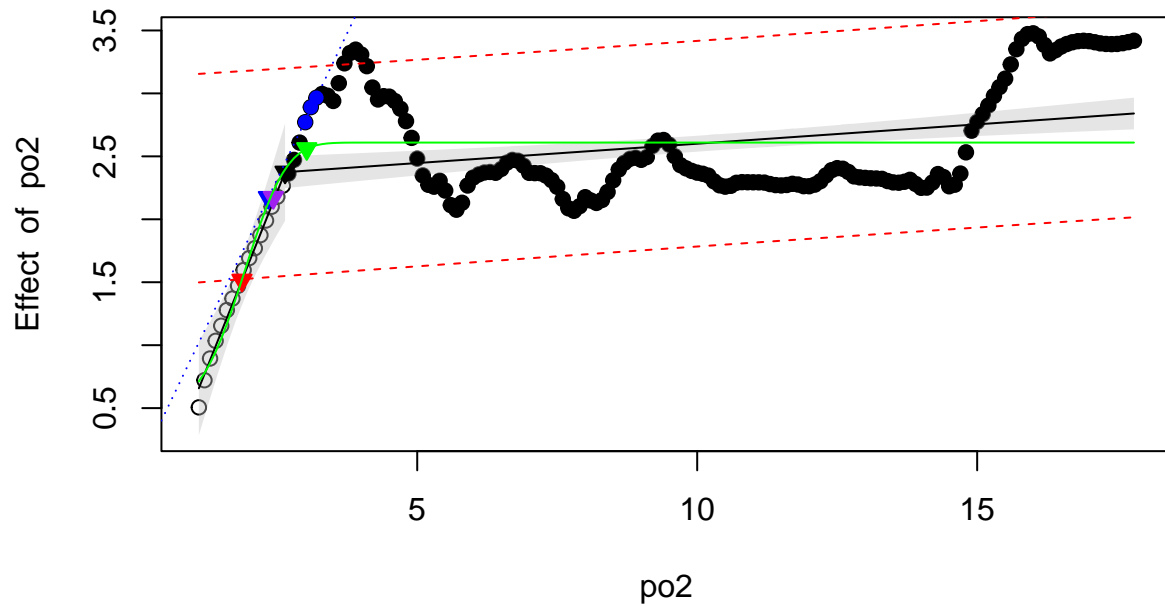
**Alpha @ MR of 2.16 = 3.363**  
**Breakpoint = 14.254**  
**LLO @ MR of 2.16 = 8.734**  
**NLR (Power) = 16.472**  
**Sub-PI = 18.1**



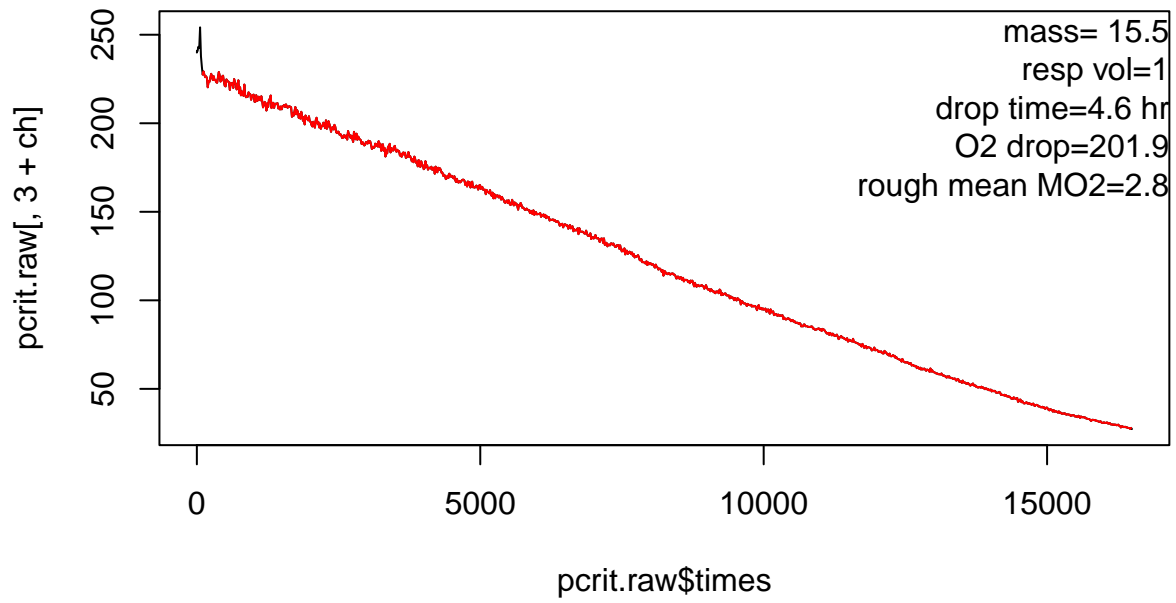
### Gr3 Muus1000-2 7 day pcrit 7-27-21.txt



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



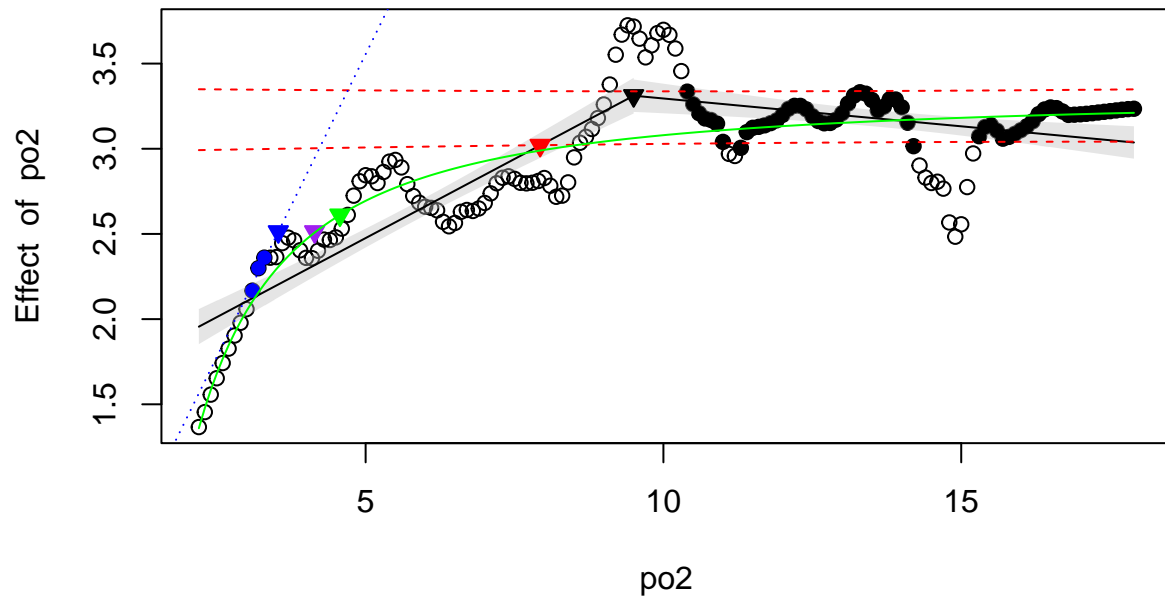
**Alpha @ MR of 2.51 = 3.535**

**Breakpoint = 9.5**

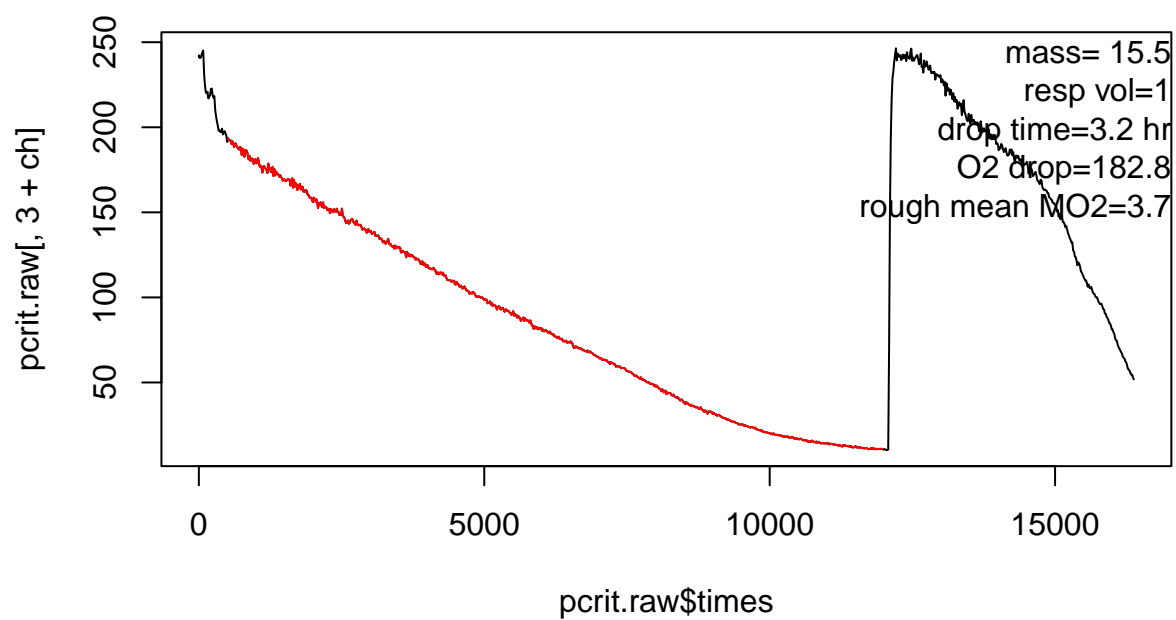
**LLO @ MR of 2.51 = 4.144**

**NLR (Pareto) = 4.569**

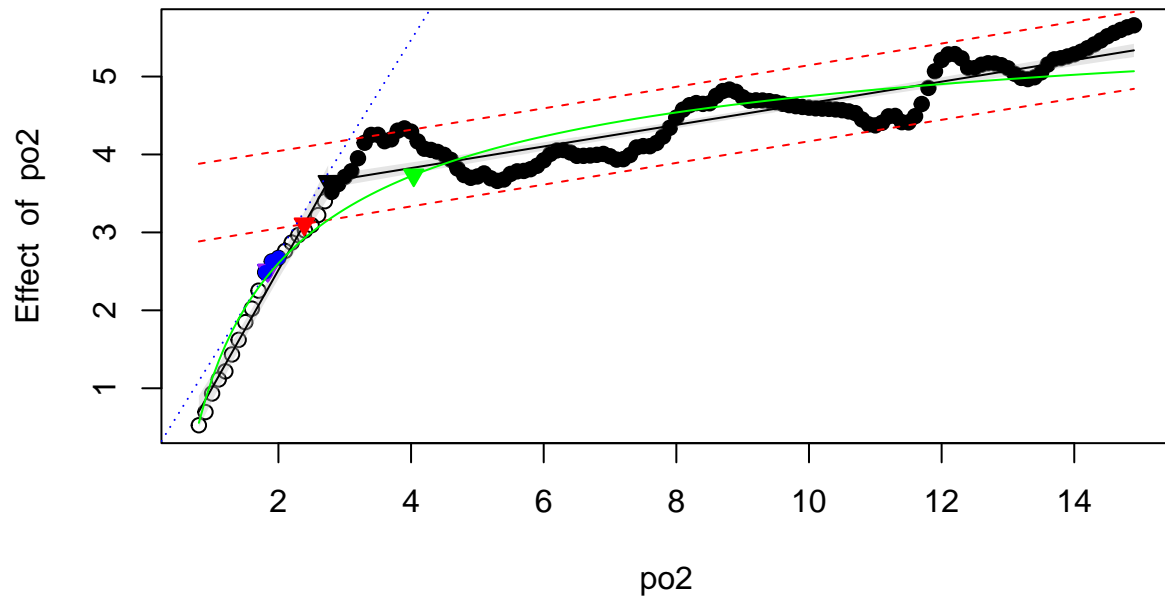
**Sub-PI = 7.93**



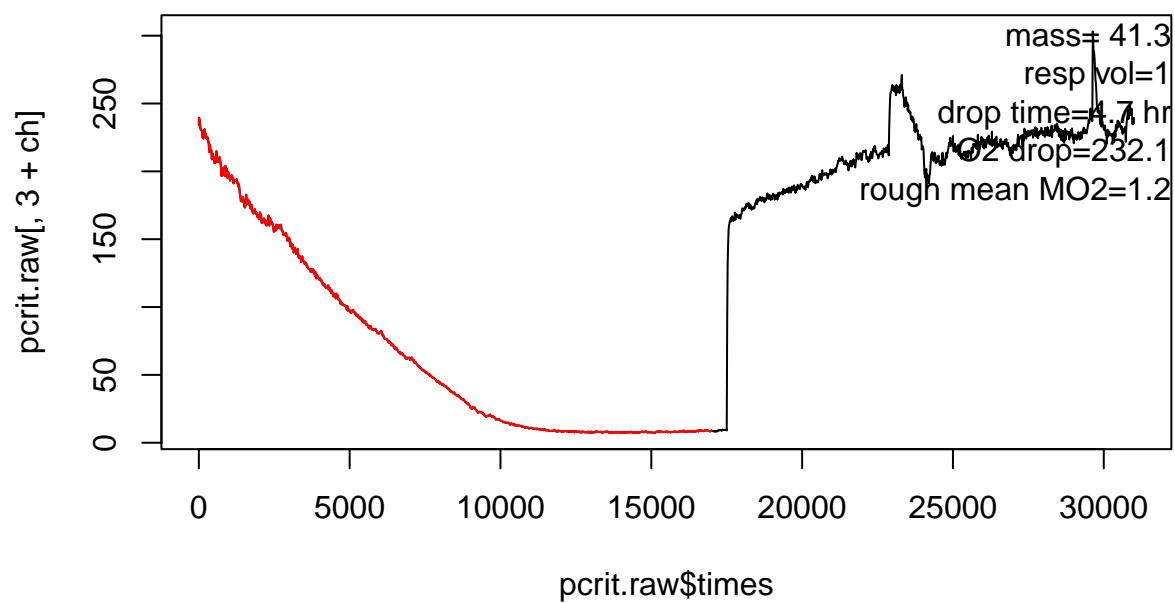
# GR4MUUS1000Pcrit-7-21-21-ch1.txt



**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**

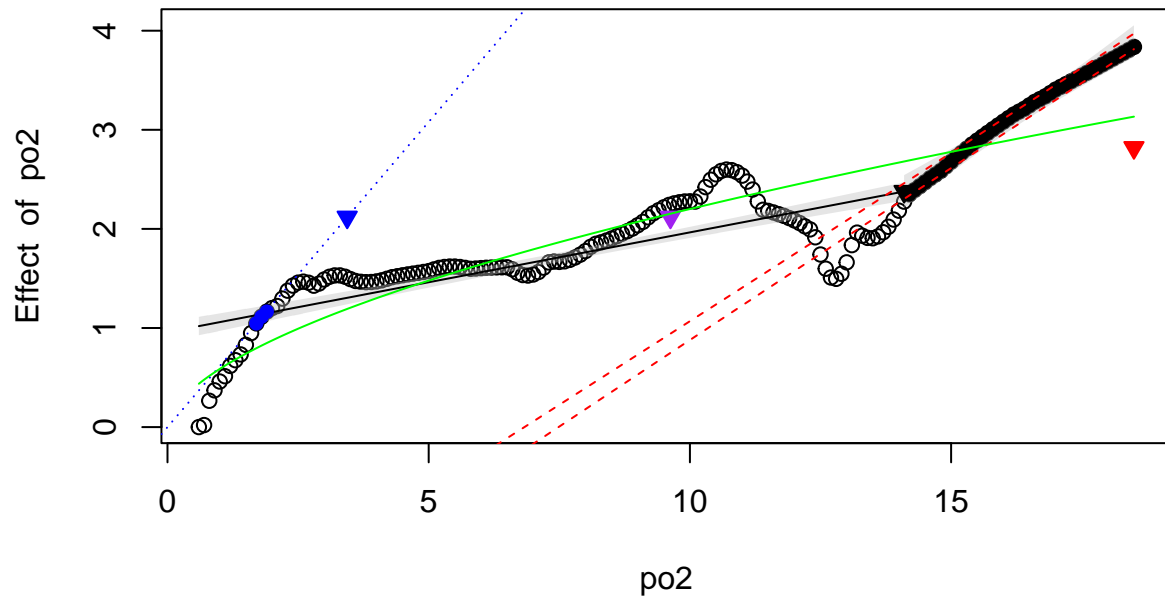


# GR4MUUS1800-2-7dayPcrit-8-3-21-ch1.txt

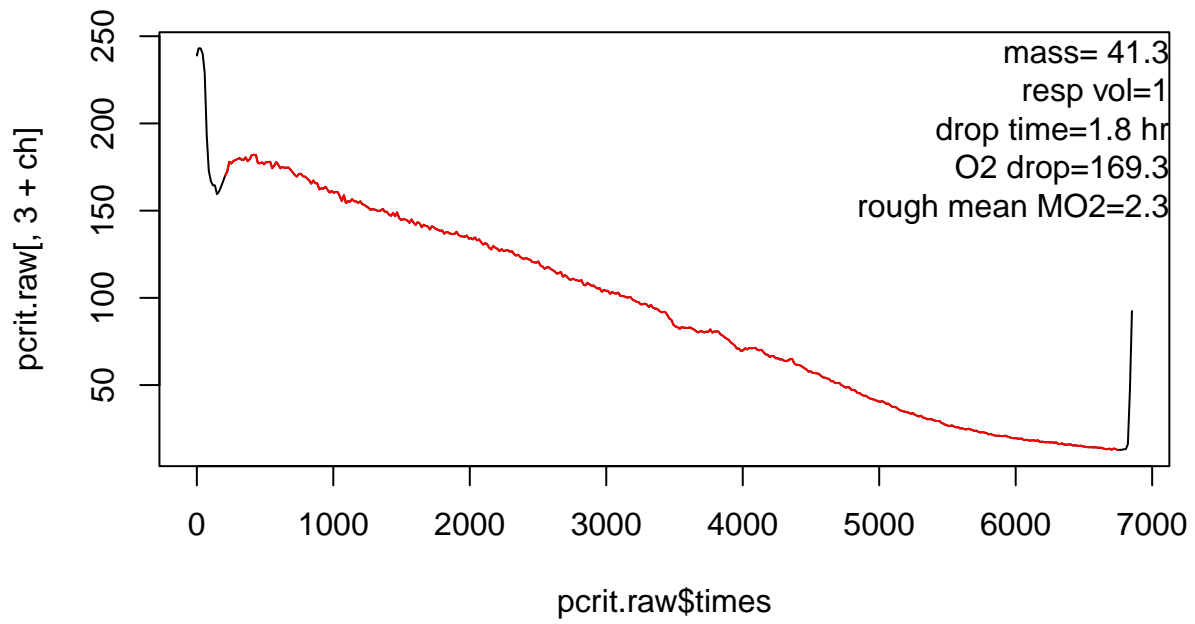




Alpha @ MR of 2.12 = 3.44  
Breakpoint = 14.1  
LLO @ MR of 2.12 = 9.627  
NLR (Power) = 38.75  
Sub-PI = 18.5



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



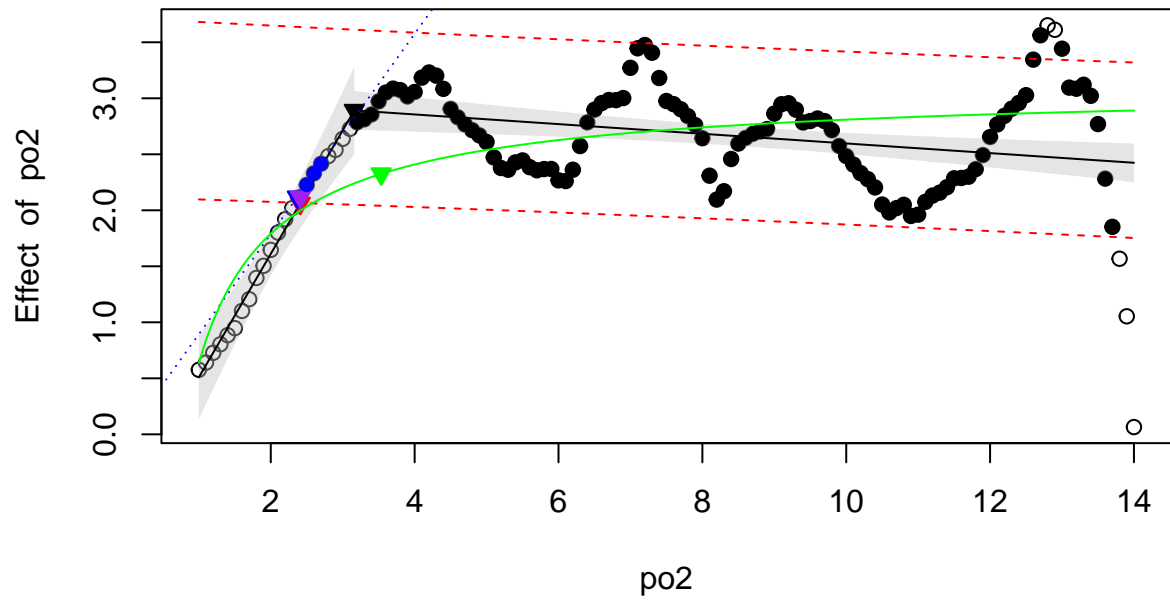
**Alpha @ MR of 2.12 = 2.37**

**Breakpoint = 3.162**

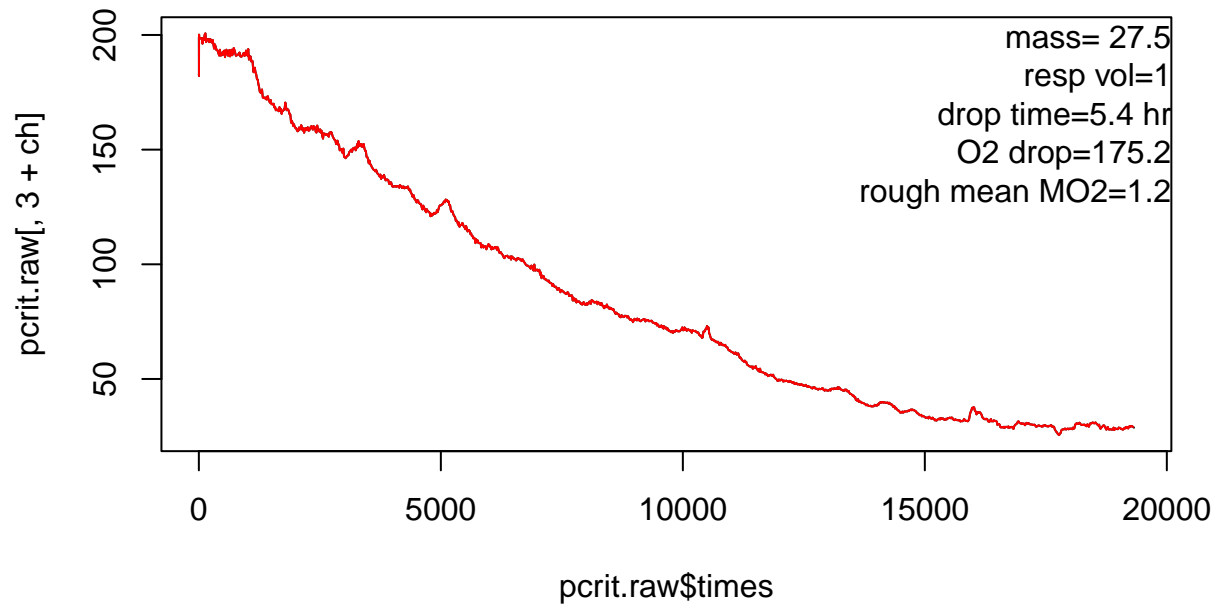
**LLO @ MR of 2.12 = 2.413**

**NLR (Pareto) = 3.535**

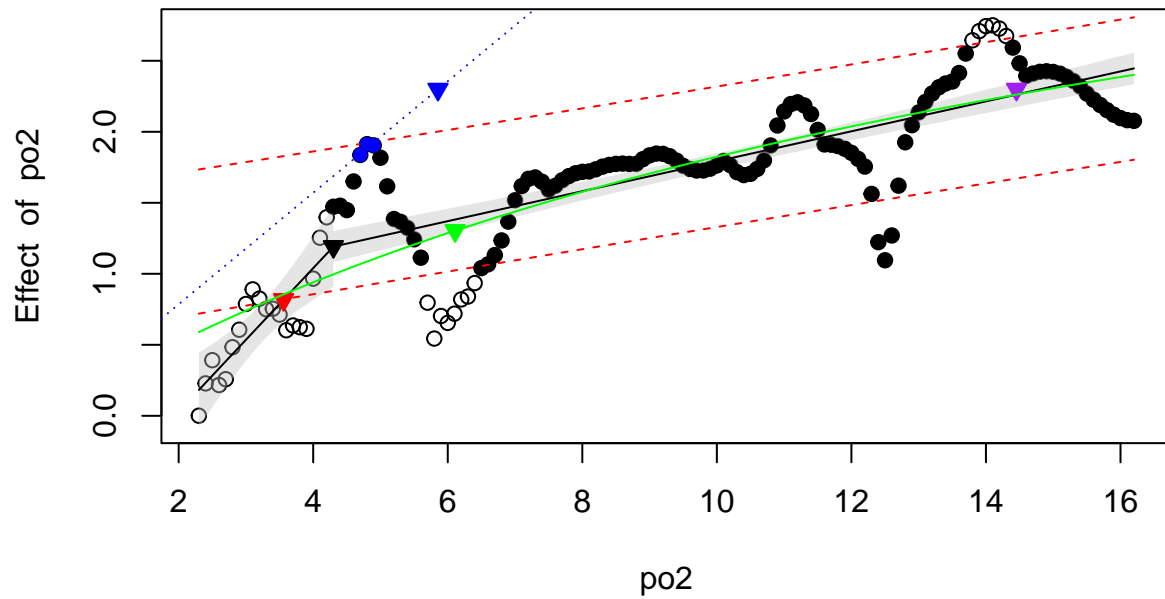
**Sub-PI = 2.41**



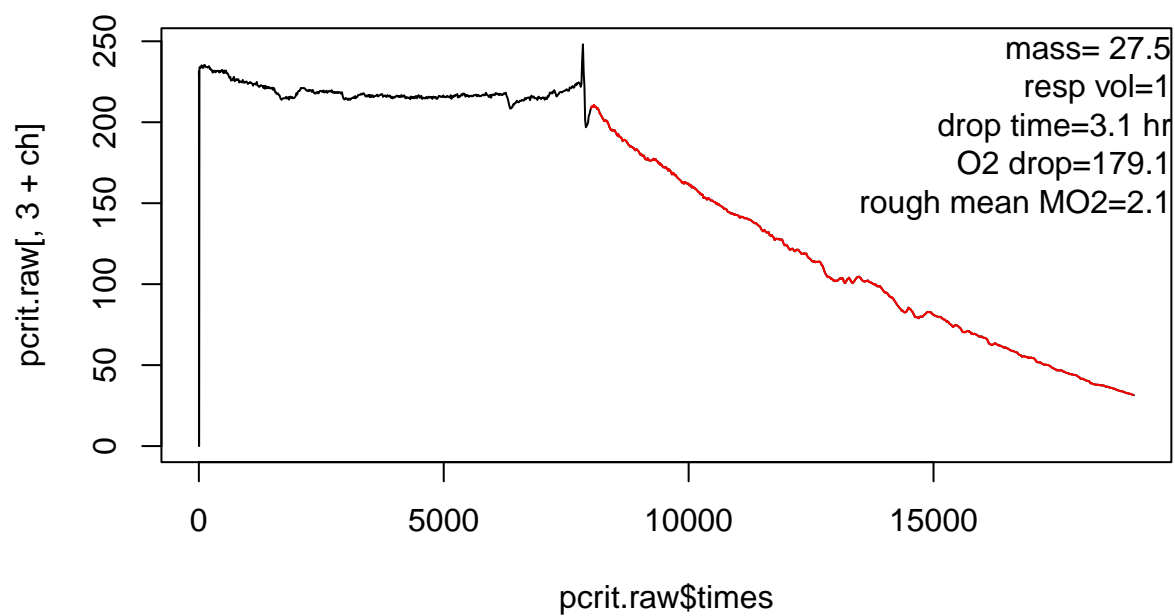
# GR4MUUS1800-7dayPcrit-7-20-21-ch1.txt



**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



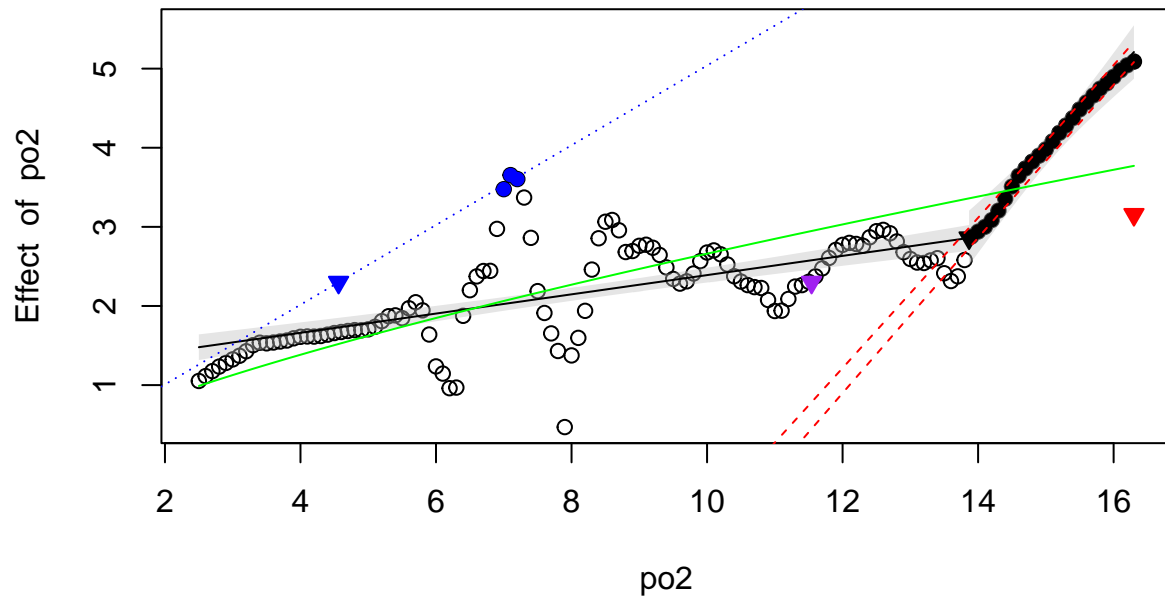
**Alpha @ MR of 2.3 = 4.562**

**Breakpoint = 13.867**

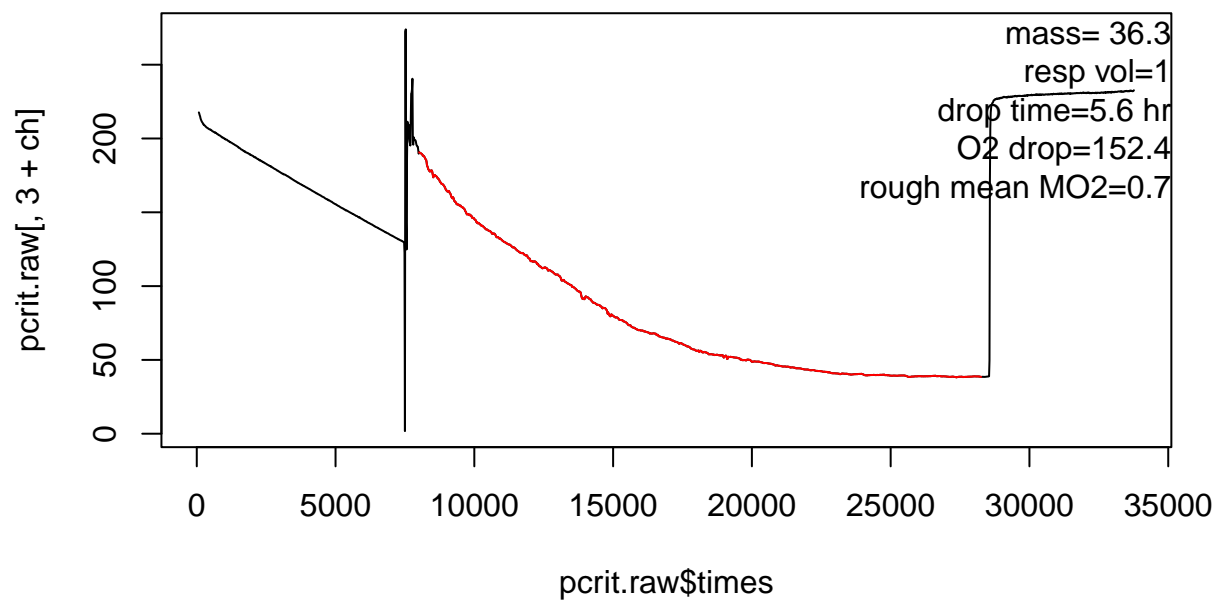
**LLO @ MR of 2.3 = 11.542**

**NLR (Power) = 37.94**

**Sub-PI = 16.3**



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





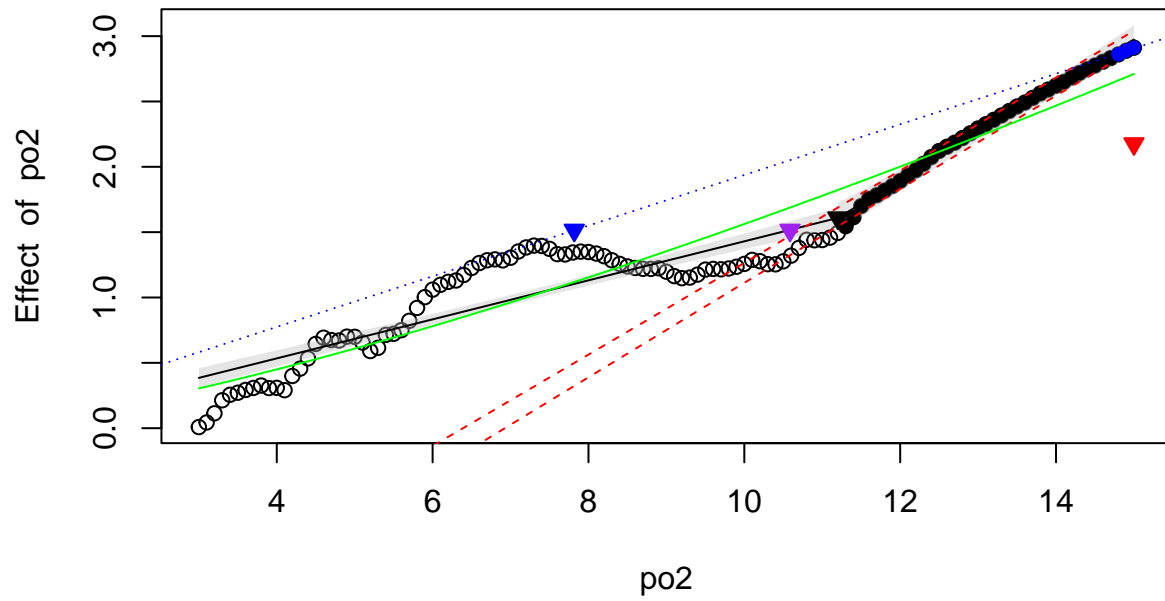
**Alpha @ MR of 1.51 = 7.818**

**Breakpoint = 11.2**

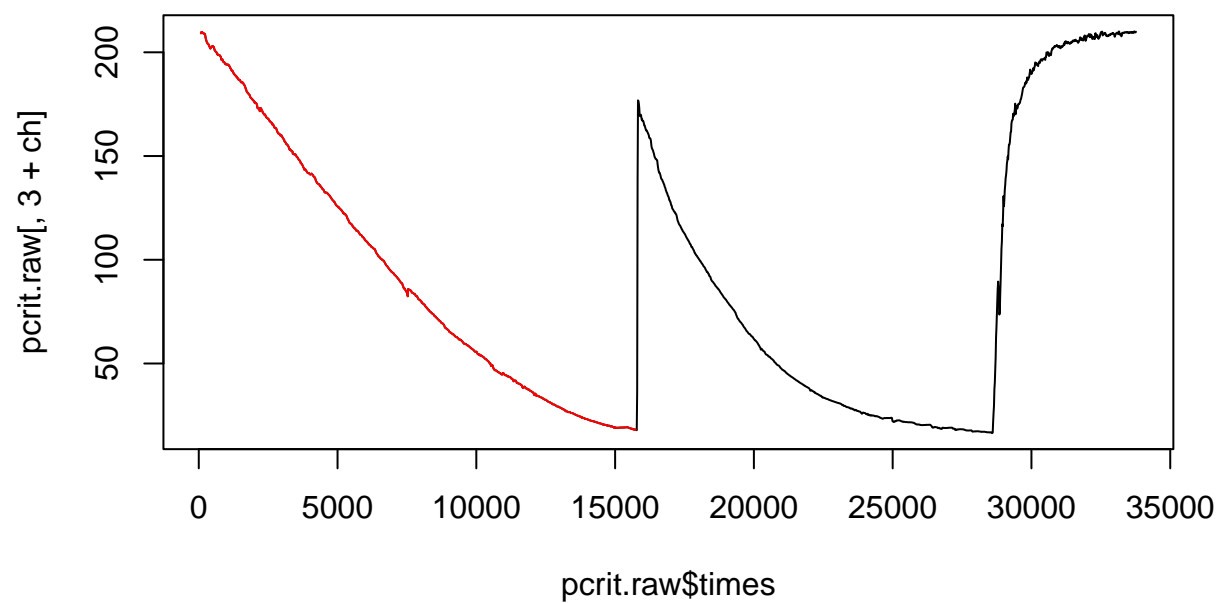
**LLO @ MR of 1.51 = 10.587**

**NLR (Power) = 28.052**

**Sub-PI = 15**



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



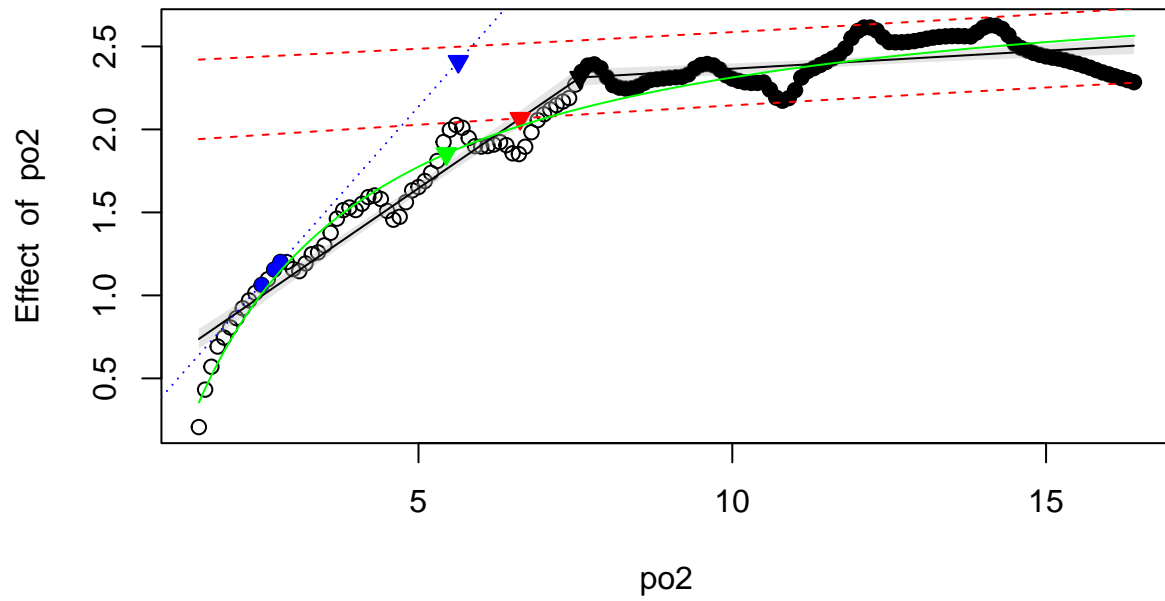
**Alpha @ MR of 2.41 = 5.635**

**Breakpoint = 7.572**

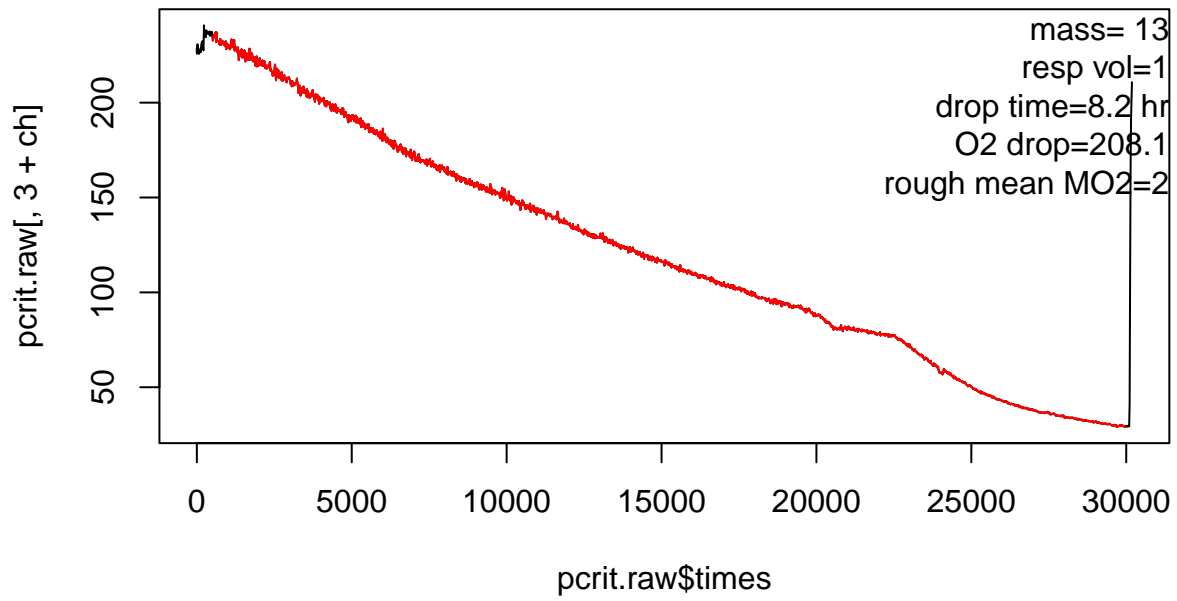
**LLO @ MR of NA = NA**

**NLR (Hyperbola) = 5.444**

**Sub-PI = 6.62**



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



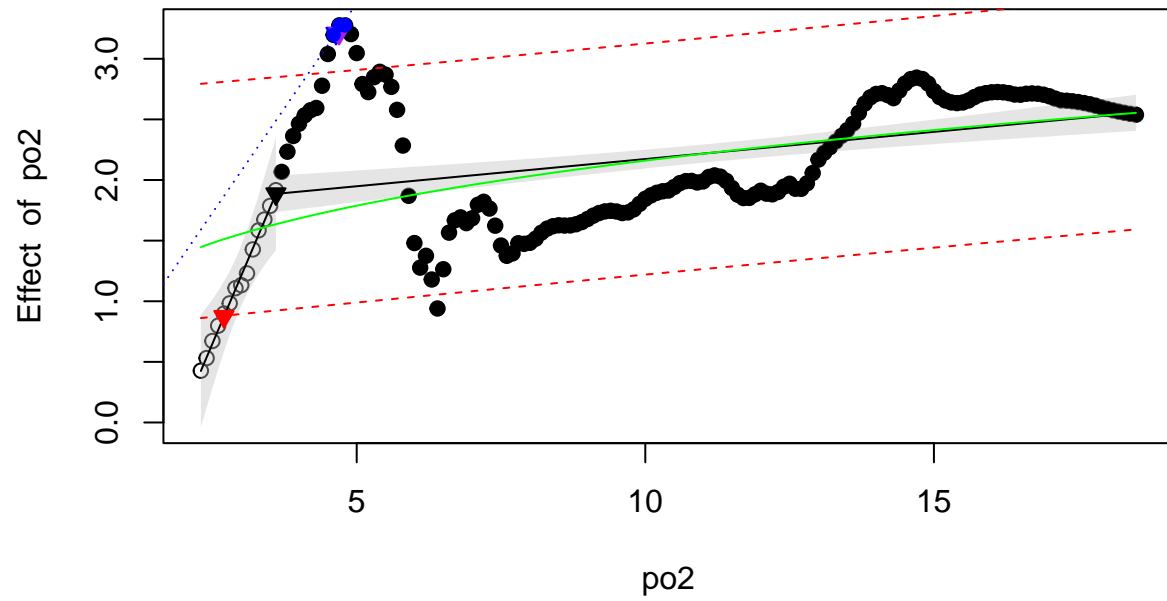
**Alpha @ MR of 3.21 = 4.642**

**Breakpoint = 3.601**

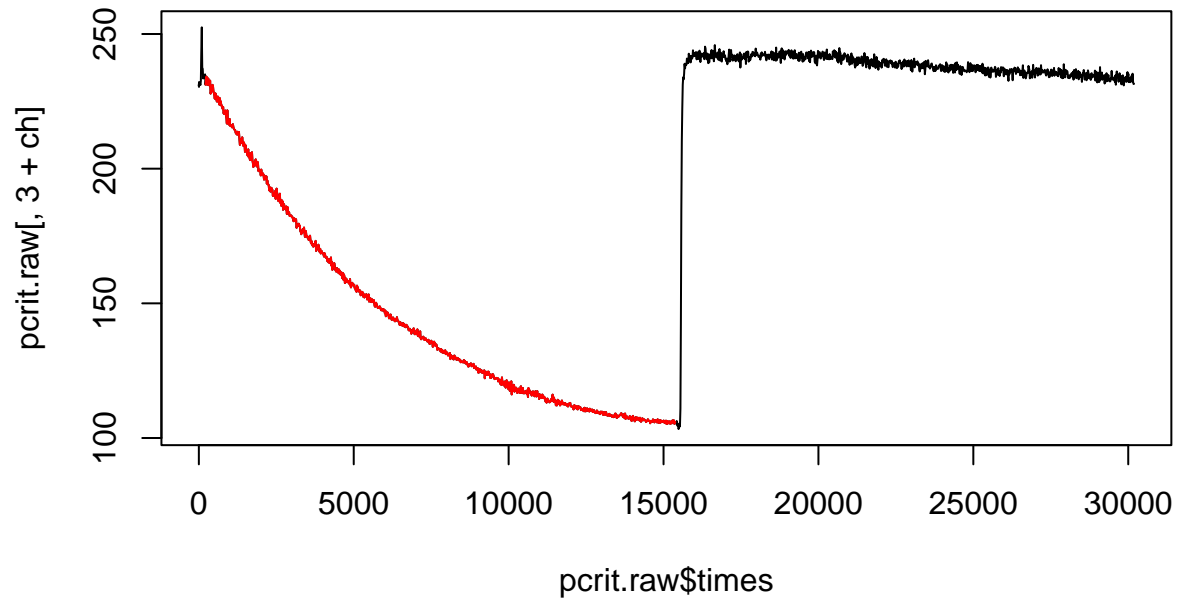
**LLO @ MR of 3.21 = 4.695**

**NLR (Power) = 77.005**

**Sub-PI = 2.7**



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



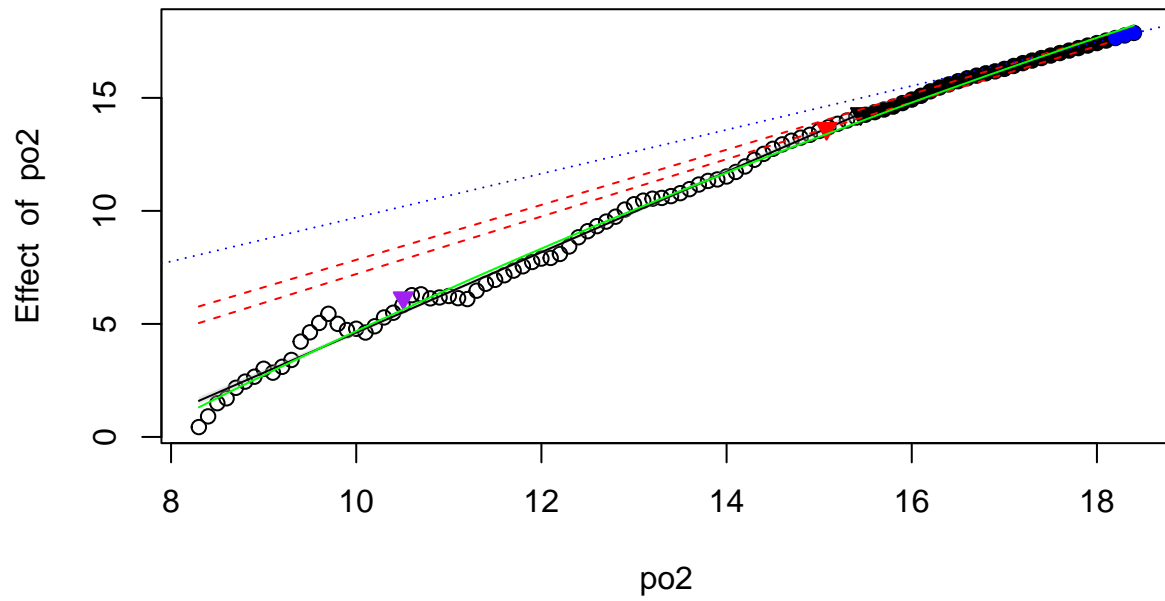
**Alpha @ MR of 6.15 = 6.337**

**Breakpoint = 15.447**

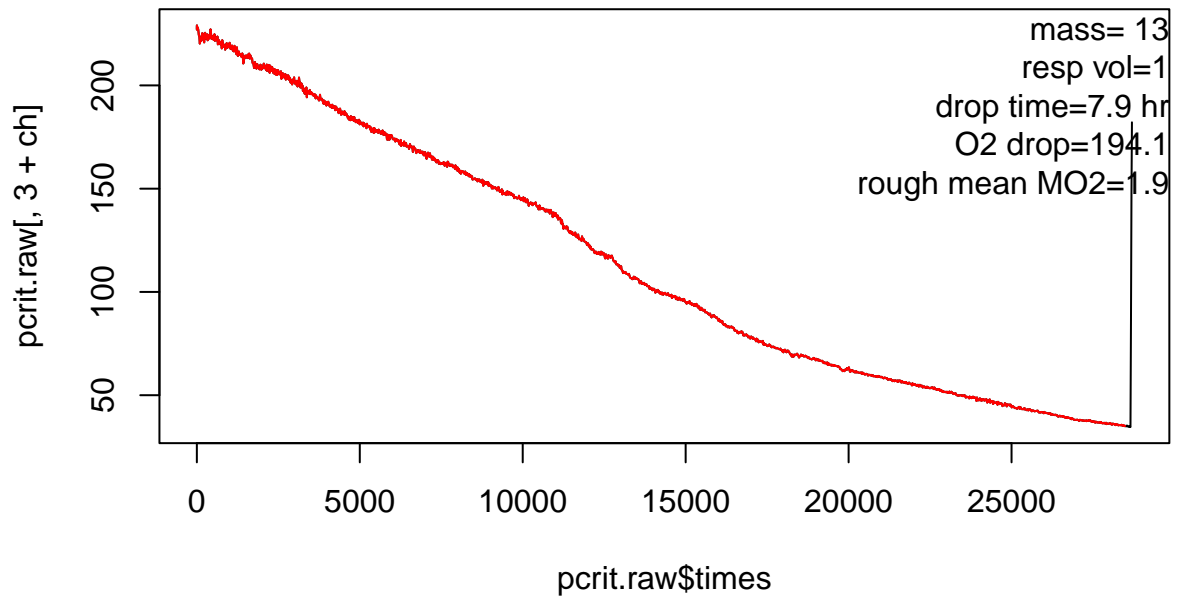
**LLO @ MR of 6.15 = 10.509**

**NLR (Hyperbola) = 24.757**

**Sub-PI = 15.08**



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





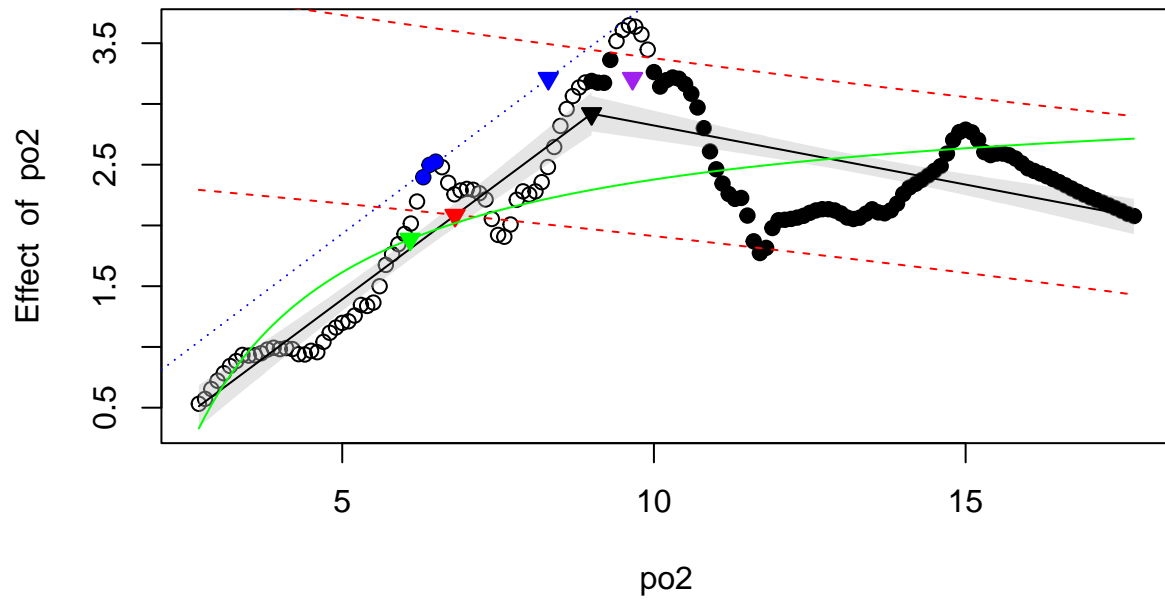
**Alpha @ MR of 3.21 = 8.306**

**Breakpoint = 9**

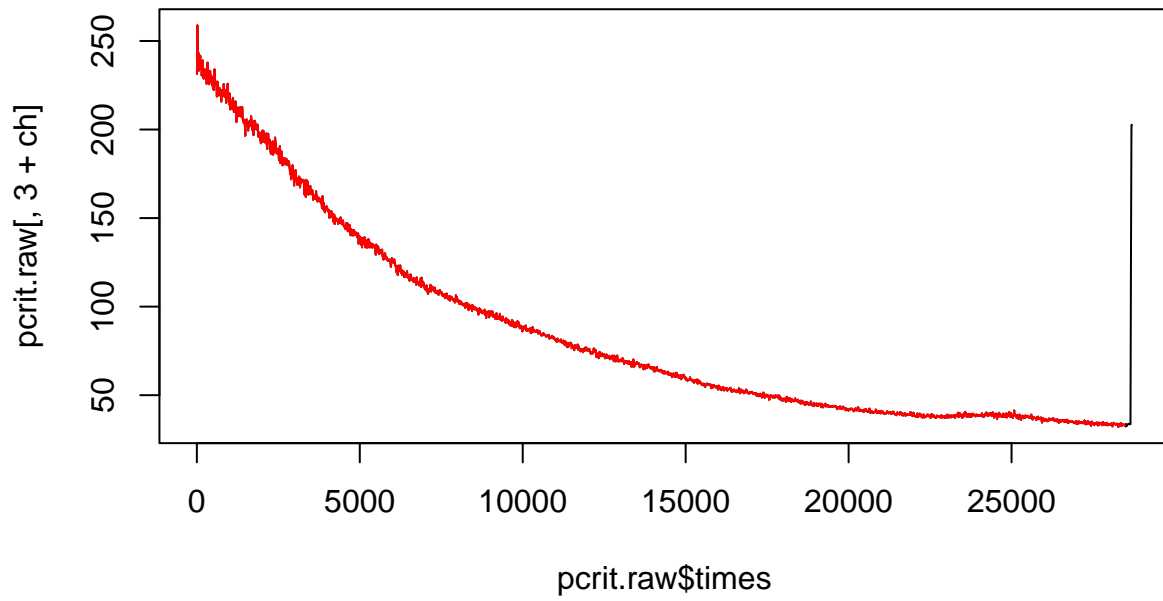
**LLO @ MR of 3.21 = 9.657**

**NLR (Pareto) = 6.088**

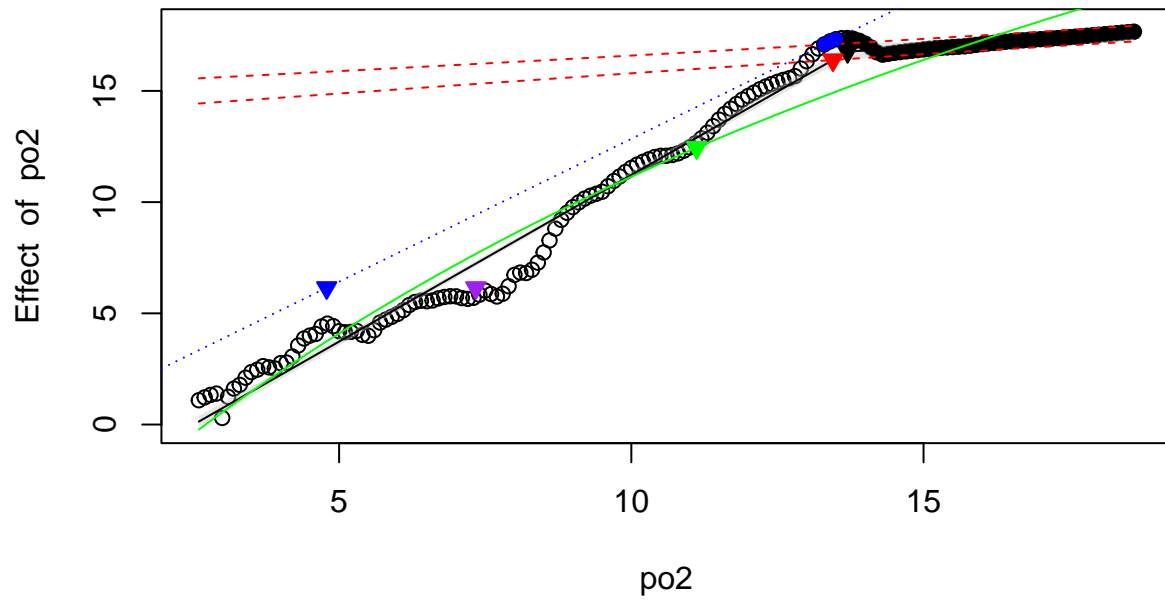
**Sub-PI = 6.81**



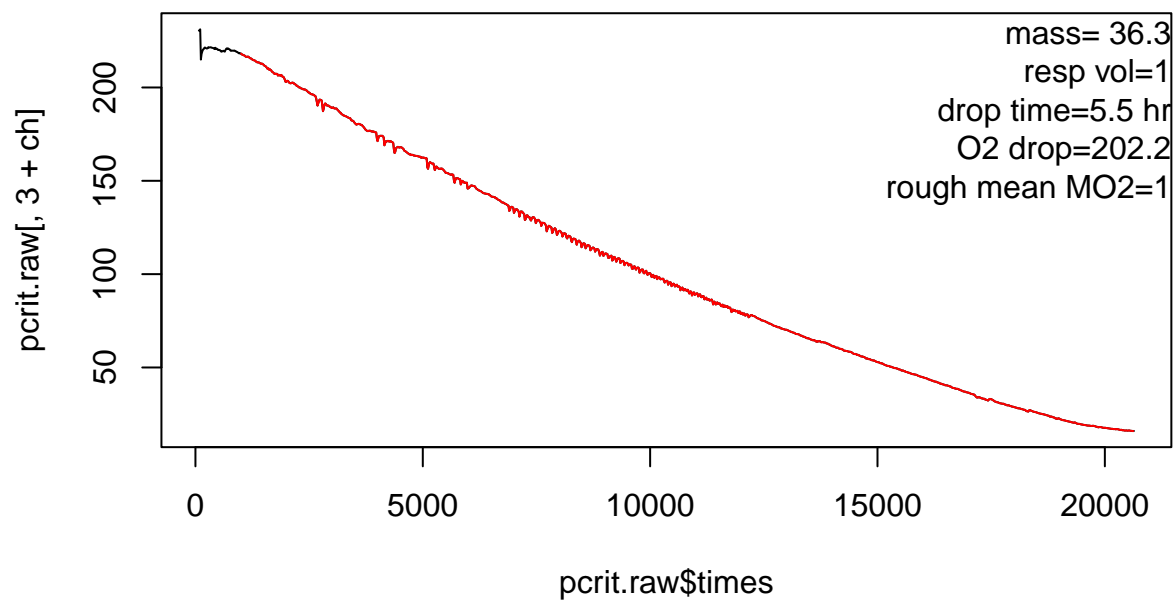
**tbocto 1000 pcrit tank 3 and 4 day 7 8-19-21-ch1.txt**



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



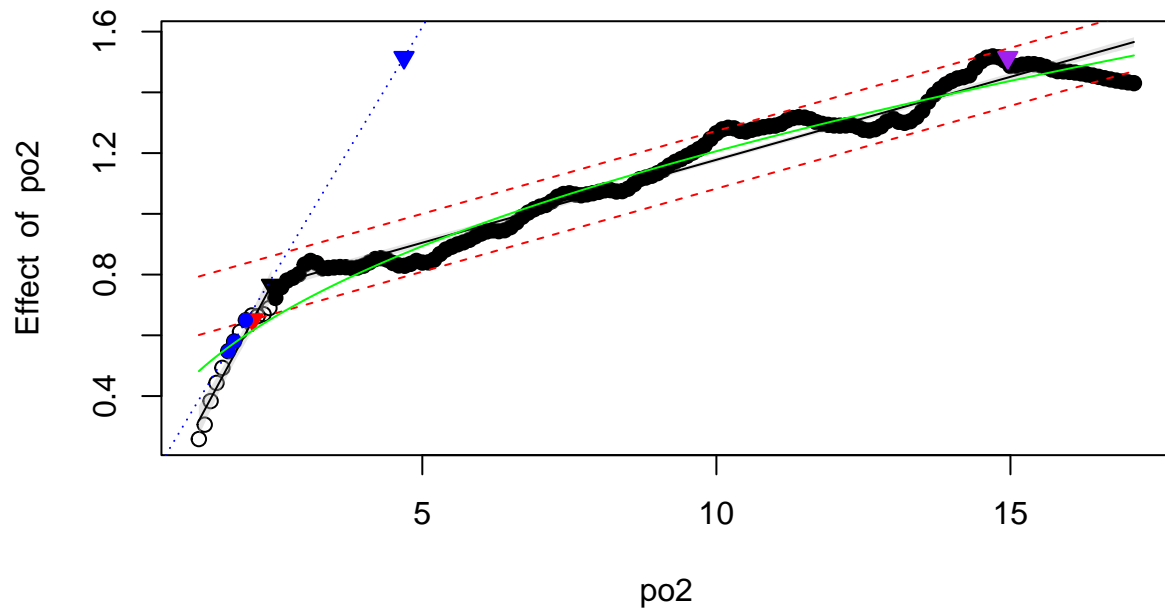
**Alpha @ MR of 1.51 = 4.689**

**Breakpoint = 2.435**

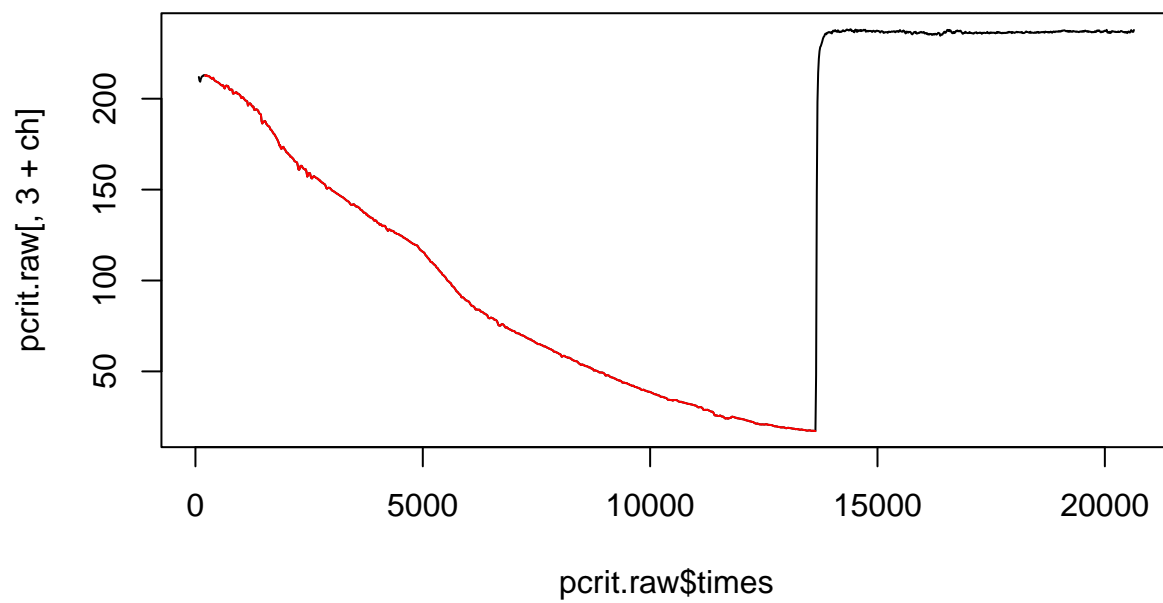
**LLO @ MR of 1.51 = 14.954**

**NLR (Power) = 66.218**

**Sub-PI = 2.12**



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



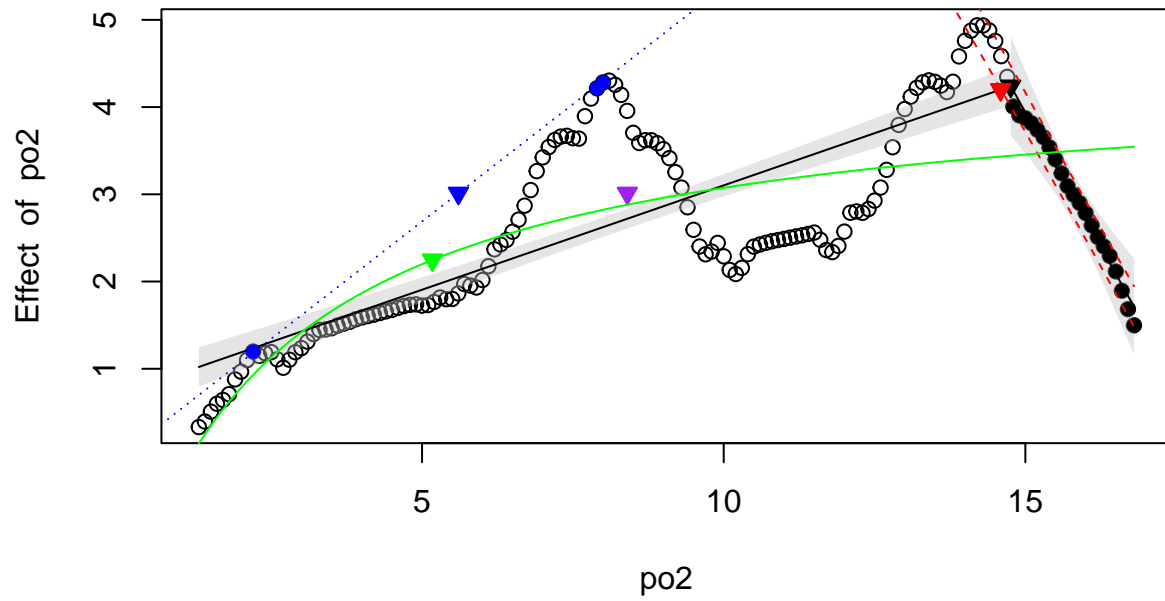
**Alpha @ MR of 3.01 = 5.603**

**Breakpoint = 14.758**

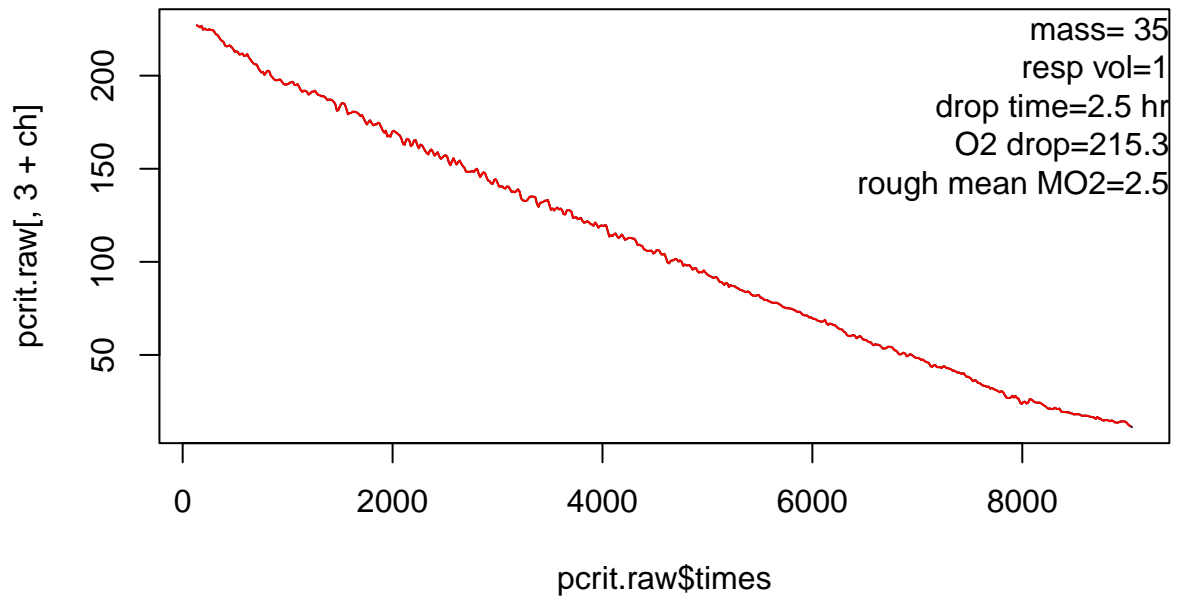
**LLO @ MR of 3.01 = 8.403**

**NLR (Hyperbola) = 5.171**

**Sub-PI = 14.59**



### gr2muus1000 pcrit 7-21-21.txt





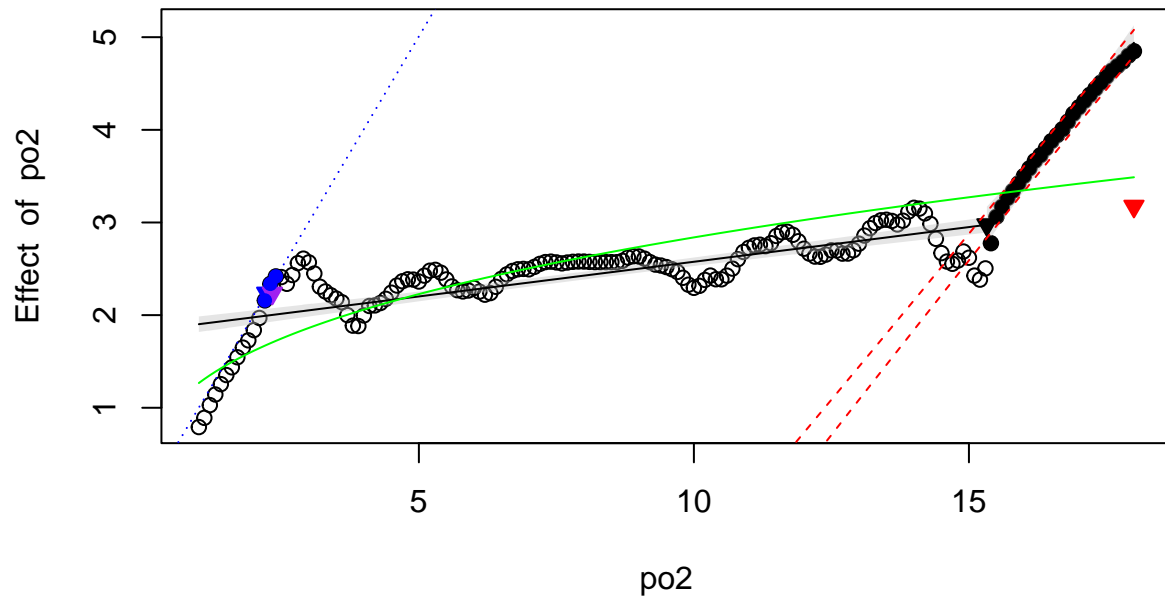
**Alpha @ MR of 2.24 = 2.233**

**Breakpoint = 15.327**

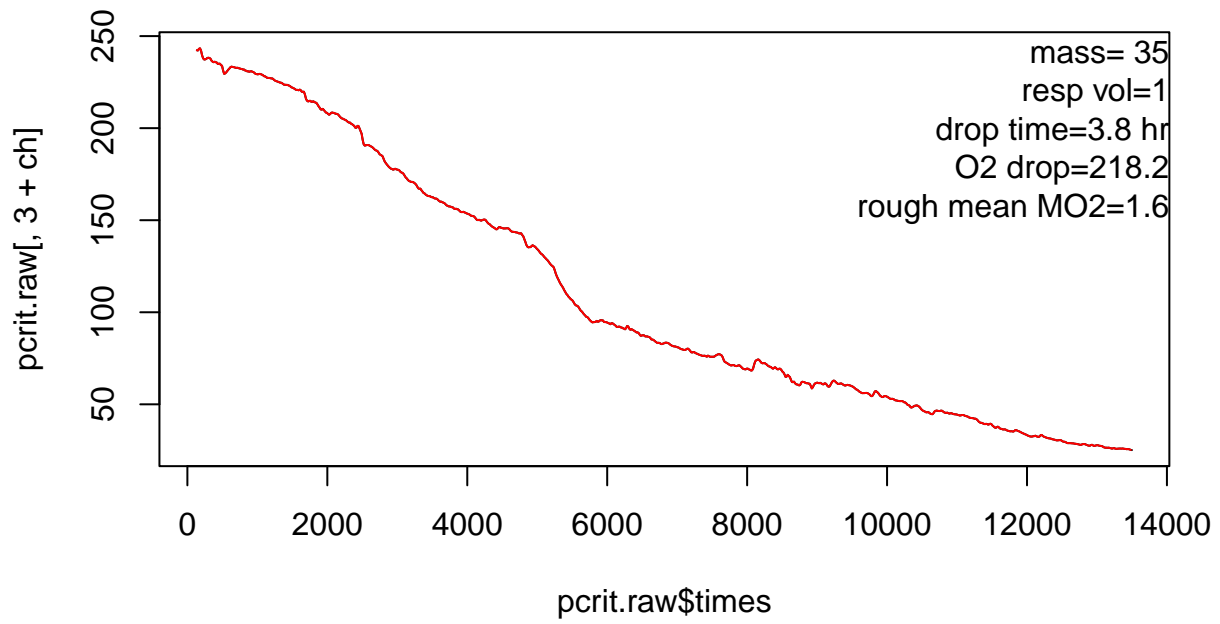
**LLO @ MR of 2.24 = 2.337**

**NLR (Power) = 64.181**

**Sub-PI = 18**



# gr2muus1000-2 pcrit 7-26-21.txt



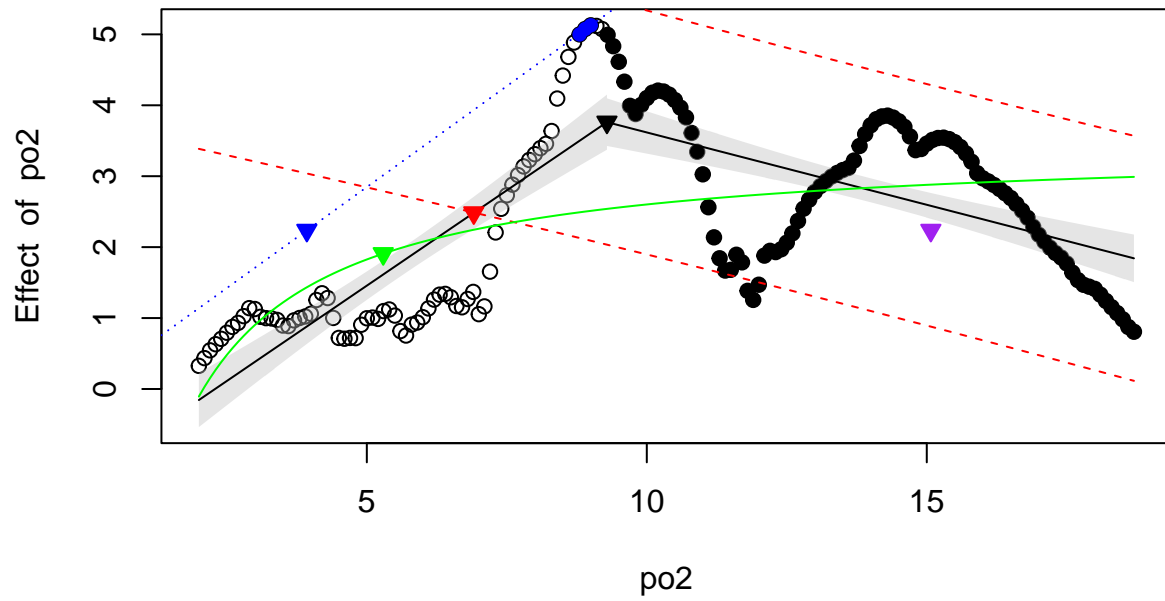
**Alpha @ MR of 2.24 = 3.929**

**Breakpoint = 9.288**

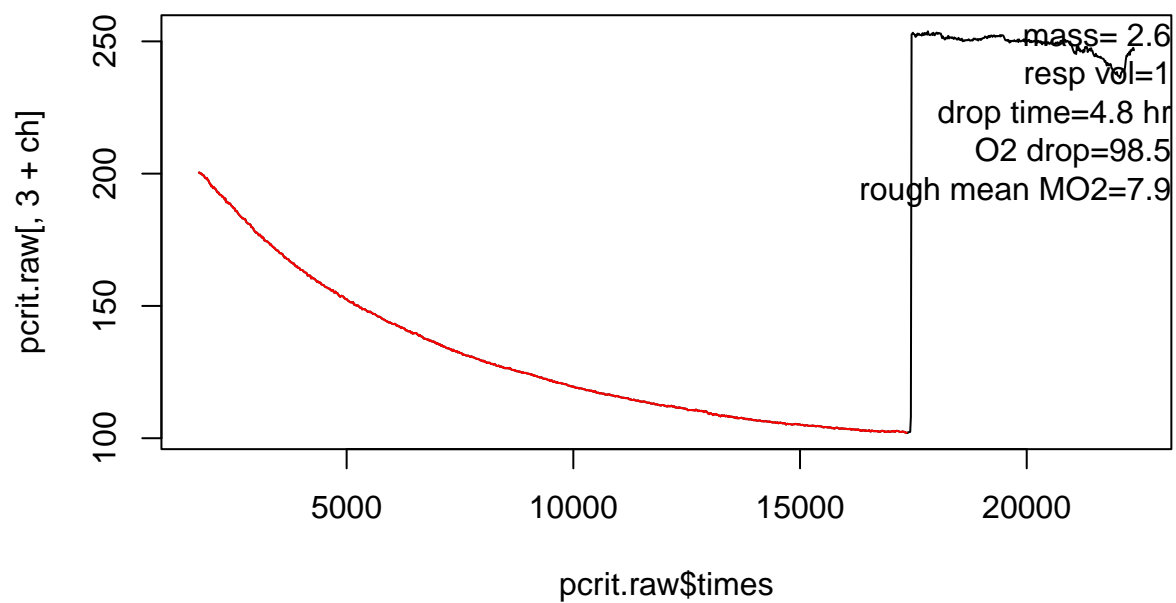
**LLO @ MR of 2.24 = 15.073**

**NLR (Hyperbola) = 5.293**

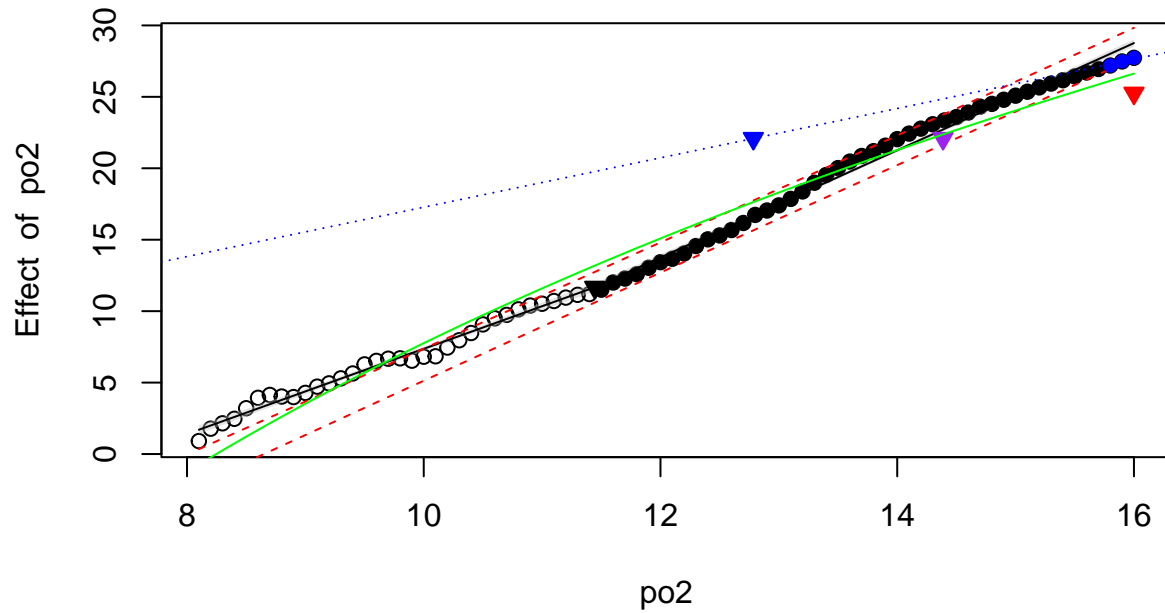
**Sub-PI = 6.91**



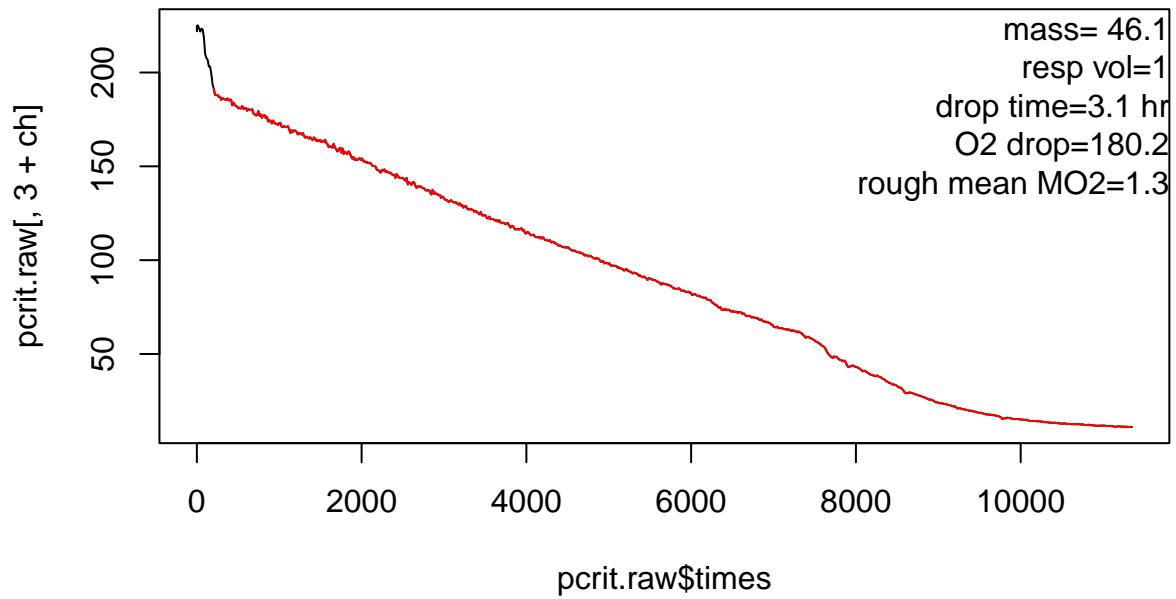
# gr2MUUS1800-2pcritday7.8-3-21.txt



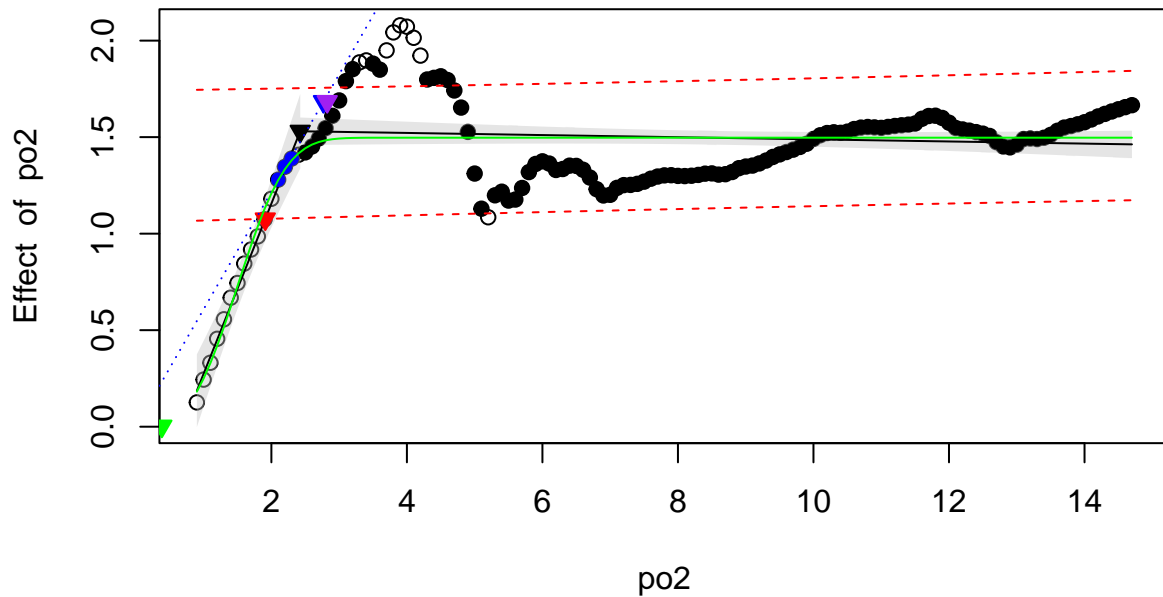
**Alpha @ MR of 22.09 = 12.785**  
**Breakpoint = 11.444**  
**LLO @ MR of 22.09 = 14.387**  
**NLR (Weibull with intercept) = 21**  
**Sub-PI = 16**



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



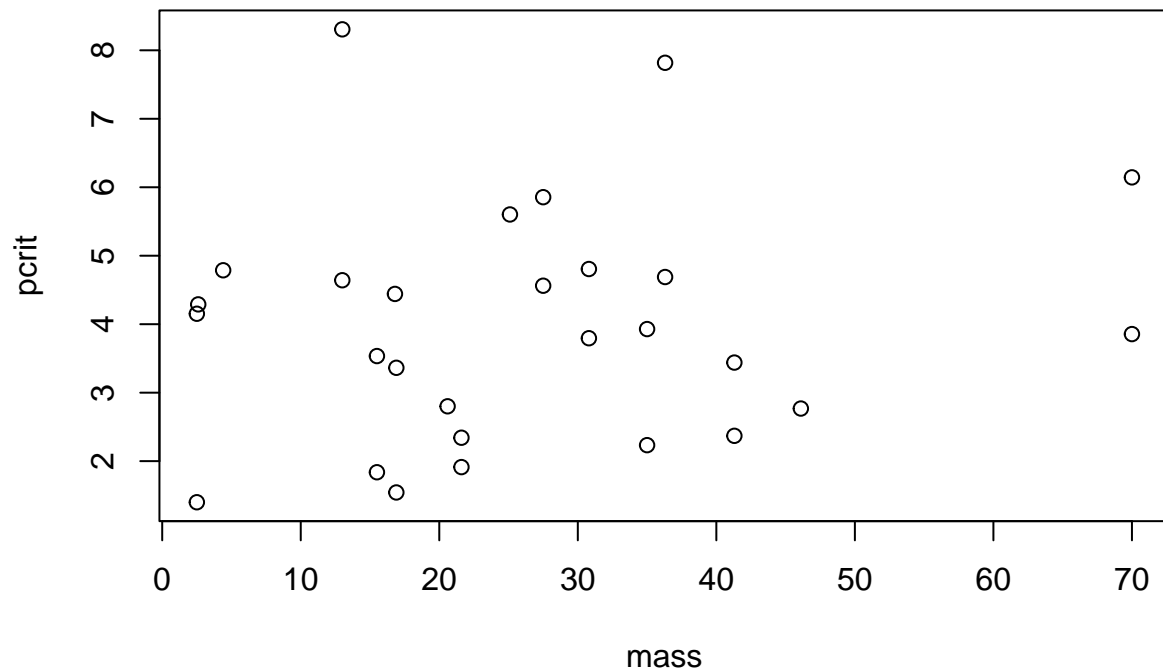
Alpha @ MR of 1.68 = 2.768  
 Breakpoint = 2.426  
 LLO @ MR of 1.68 = 2.824  
 NLR (Weibull with intercept) = 0.384  
 Sub-PI = 1.91



Next, I remove the measurements for which the data was excluded (runs did not reach oxygen level of 50 mmHgO<sub>2</sub>). 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 Plotting the Critical Oxygen Pressure results

```
svg(filename = "Figure_2.svg",width=3.5,height=3.5,pointsize=5)
par(fig=c(0.05,1,0.03,1))
boxplot(pcrit~day+pco2,data=pcrits,range=0,
        axes=F,col=c("grey75","grey40","grey75","grey40"),ylab="",xlab="")
box()
abline(v=2.5)
axis(1, at=c(1.5,3.5),labels = c(1000,1800),tick=F,cex.axis=2)
axis(2,at=c(2,4,6,8),cex.axis=2)
mtext(expression("Critical Oxygen Pressure (kPa)"),side=2,cex=2.7,line=3)
mtext(expression("pCO"[2]*" ("*mu*"atm)"),side=1,cex=2.7,line=4)
legend(3.5,8.5,pt.bg=c("grey75","grey40"),legend=c("Day 1","Day 7"),
      pch=22,cex=2,pt.cex=5,bty="n")
dev.off()

## pdf
## 2
```

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

```
cairosvg Figure_2.svg -o Figure_2.png -d 300
```

Converting to eps for submission.

```
inkscape Figure_2.svg -o Figure_2.eps --export-ignore-filters --export-ps-level=3
```

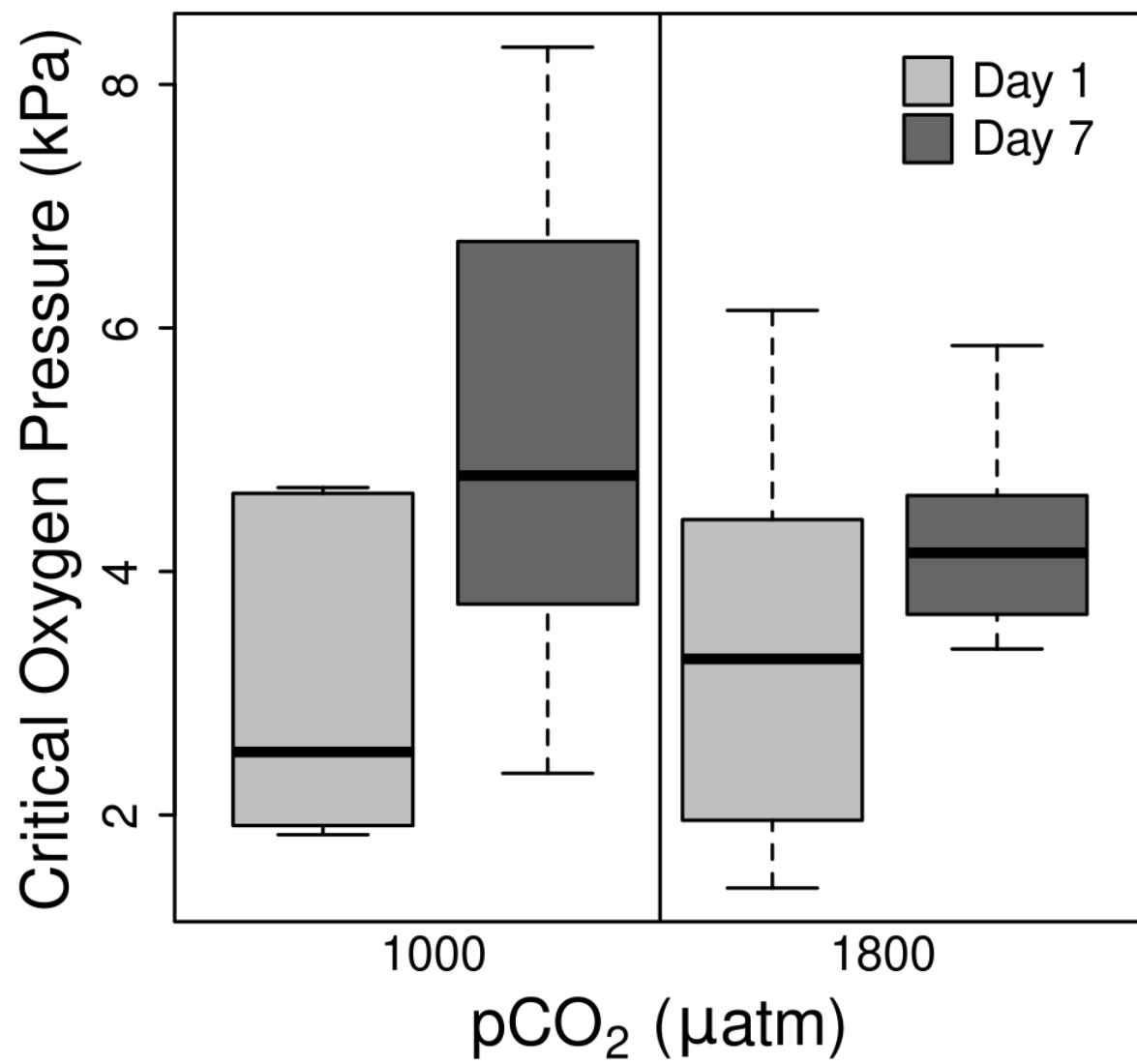


Figure 1: work in progress text