

Supplementary Material

Population thermal regime modulates the response of the agarophyte *Gelidium corneum* to marine heatwaves

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Growth (RGR): Main axis elongation

1. DATA PROCESSING

Data loading and inspection. First of all, we will visualize raw data using a boxplot.

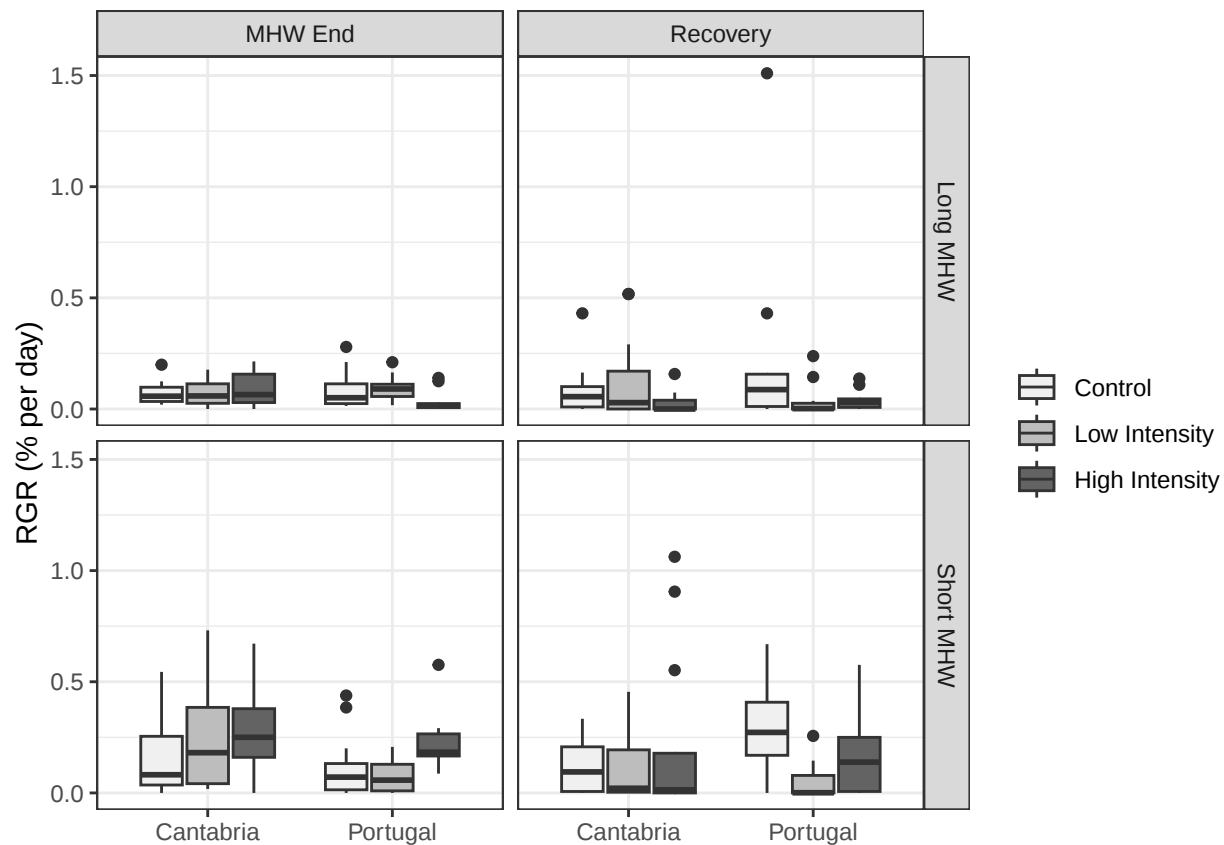
```
# Data
Length<-data.frame(read.xlsx("D:/Estancia_Porto_2023/experiments/MHW/Full_Datasets.xlsx",
                             sheet = 2))
Length$Growth<-(((Length$End - Length$Start)/Length$Start)/Length$Days)*100
Length<-na.omit(Length)

# Labels
day.labs <- c("MHW End", "Recovery")
names(day.labs) <- c("MHW", "Recovery")
Duration.labs <- c("Short MHW", "Long MHW")
names(Duration.labs) <- c("S.Rec", "L.Rec")
MHW.labs <- c("Control", "Low Intensity", "High Intensity")

# Raw boxplot
Length_plot <- ggplot(Length, aes(x = as.factor(Population), y = Growth,
                                     fill = as.factor(Heat_Treatment))) +

  geom_boxplot() +
  facet_grid(Duration_Treatment~Phase,
             labeller = labeller(Duration_Treatment = Duration.labs,
                                   Phase = day.labs)) +
  labs(x = NULL, y = 'RGR (% per day)', fill = NULL) +
  theme(text=element_text(family=windowsFont("Arial")),
        axis.text.x = element_text(color = "grey20", size = 14),
        axis.text.y = element_text(color = "grey20", size = 14),
        axis.title.x = element_text(color = "grey20", size = 16),
        axis.title.y = element_text(color = "grey20", size = 16),
        strip.text.x = element_text(color = "grey20", size = 16),
        strip.text.y = element_text(color = "grey20", size = 16),
        legend.text = element_text(color = "grey20", size = 14),
        legend.background = element_rect(colour = "grey20", fill = "white", size = 0.3)) +
  scale_fill_brewer(palette="Greys", labels = MHW.labs) + theme_bw()

Length_plot
```



```
# Prepare data for statistical analysis
```

```
Length_Stats <- Length
Length_Stats$ID <- as.factor(Length_Stats$ID)
Length_Stats$Phase <- as.factor(Length_Stats$Phase)
Length_Stats$Tank <- as.factor(Length_Stats$Tank)
Length_Stats$Heat_Treatment <- as.factor(Length_Stats$Heat_Treatment)
Length_Stats$Population <- as.factor(Length_Stats$Population)
Length_Stats$Growth_Trif <- log10(Length_Stats$Growth+1)
summary(Length_Stats)
```

```
##      Population Heat_Treatment Duration_Treatment      Phase
## Cantabria:144   Control:96      Length:288         MHW      :144
## Portugal :144   MHW2 :96      Class :character    Recovery:144
##                      MHW4 :96      Mode  :character
##
##
##
##      Days      Tank      ID      Replicate      Plant
## Min. : 7.0      1       : 24      1       : 4      Length:288      Min. : 1.00
## 1st Qu.: 7.0      2       : 24      2       : 4      Class :character 1st Qu.: 3.75
## Median : 7.0      3       : 24      3       : 4      Mode  :character Median : 6.50
## Mean :10.5      4       : 24      4       : 4
## 3rd Qu.:10.5      5       : 24      5       : 4
## Max. :21.0      6       : 24      6       : 4
##                      (Other):144 (Other):264
```

```
##      Start      End      Growth      Growth_Trif
## Min.    : 7.724   Min.    : 7.872   Min.    :0.000000   Min.    :0.000000
## 1st Qu.:11.601   1st Qu.:11.669   1st Qu.:0.009474   1st Qu.:0.004095
## Median :13.031   Median :13.118   Median :0.062833   Median :0.026465
## Mean   :13.056   Mean   :13.199   Mean   :0.128358   Mean   :0.047799
## 3rd Qu.:14.527   3rd Qu.:14.655   3rd Qu.:0.171217   3rd Qu.:0.068637
## Max.    :17.826   Max.    :17.985   Max.    :1.510051   Max.    :0.399683
##
```

```
Length_Stats_SMHW <- Length_Stats[Length_Stats$Duration_Treatment == "S.Rec",]
Length_Stats_LMHW <- Length_Stats[Length_Stats$Duration_Treatment == "L.Rec",]
```

2. STATISTICAL ANALYSIS

To test the effects of the different treatments (Long/Short, Low/High Intensity MHW) on the relative growth rate (RGR) we follow a factorial design with repeated measures. The analysis was performed separately for each MHW (Long/Short) as the results were compared to independent control treatments. In this context, **POPULATION** (w/ two levels: Cantabrian and Atlantic) and **MHW INTENSITY** (w/ three levels: Control, Low Intensity and High intensity) were considered between-subject factors, while **TIME** (w/ two levels: after MHW and after recovery period) was considered a within-subject factor. The response variable measured was the **RGR**. The aim was to understand how RGR changes over time (After Stress Period vs Recovery) depending on the population of origin and the induced thermal stress imposed by the MHW (Low vs High vs Non-Stress).

2.1. Check model assumptions

2.1.1. Outliers

First of all, the outliers were identified and removed when considered extreme.

```
# Large MHW
outliers_LMHW <- Length_Stats_LMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Growth_Trif)
outliers_LMHW <- outliers_LMHW[-c(13,14)]
Length_Stats_LMHW_NO <- anti_join(Length_Stats_LMHW, outliers_LMHW, by = c("ID"))
outliers_LMHW %>%
  select(Phase, Population, Heat_Treatment, Duration_Treatment, ID, Growth) %>%
  head() # Large MHW outliers
```

```
## # A tibble: 6 x 6
##   Phase      Population Heat_Treatment Duration_Treatment ID      Growth
##   <fct>      <fct>      <fct>          <chr>          <fct>    <dbl>
## 1 Recovery Cantabria Control        L.Rec          58      0.430
## 2 Recovery Cantabria MHW2          L.Rec          31      0.517
## 3 Recovery Cantabria MHW2          L.Rec          33      0.517
## 4 Recovery Cantabria MHW4          L.Rec           5      0.158
## 5 MHW        Portugal Control        L.Rec          64      0.279
## 6 Recovery Portugal Control        L.Rec          63      0.430
```

```
# Short MHW
outliers_SMHW <- Length_Stats_SMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Growth_Trif)
outliers_SMHW <- outliers_SMHW[-c(13,14)]
Length_Stats_SMHW_NO <- anti_join(Length_Stats_SMHW, outliers_SMHW, by = c("ID"))
outliers_SMHW %>%
  select(Phase, Population, Heat_Treatment, Duration_Treatment, ID, Growth) %>%
  head() # Large MHW outliers
```

```
## # A tibble: 6 x 6
##   Phase      Population Heat_Treatment Duration_Treatment ID      Growth
##   <fct>      <fct>      <fct>          <chr>          <fct>  <dbl>
## 1 Recovery Cantabria MHW4           S.Rec           2      0.905
## 2 Recovery Cantabria MHW4           S.Rec           8      1.06
## 3 Recovery Cantabria MHW4           S.Rec           9      0.552
## 4 MHW        Portugal Control        S.Rec           68     0.438
## 5 MHW        Portugal Control        S.Rec           70     0.385
## 6 Recovery Portugal MHW2           S.Rec           45     0.257
```

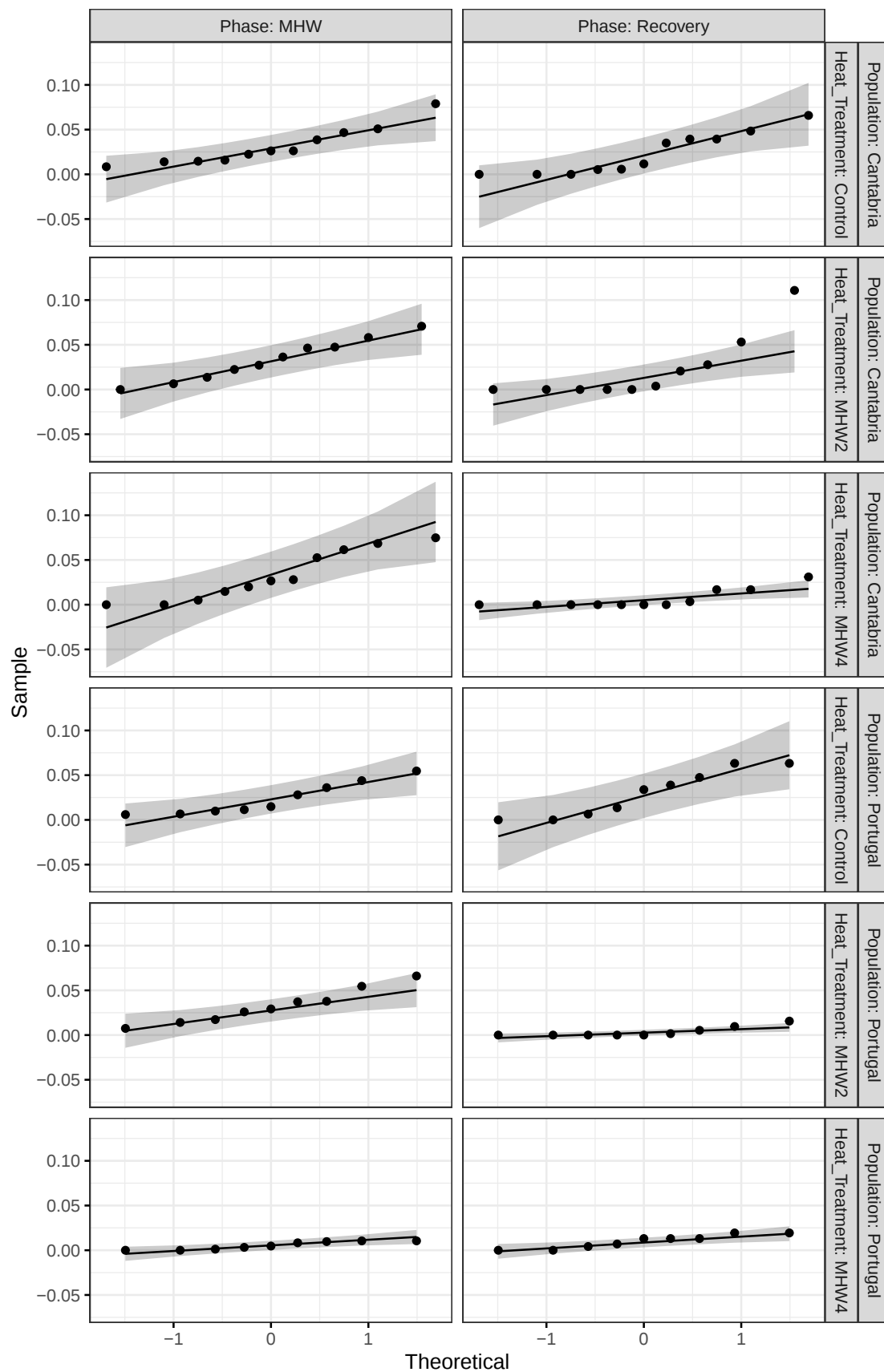
2.1.2. Normality

The second step was to test normality. For that purpose, we computed Shapiro-Wilk's tests for each combinations of factor levels. Normality was also assessed visually by inspecting qqplots. Shapiro-Wilk's tests revealed normality issues, which were also identified in qqplots. Data transformations (log10 transf.) did not improve normality.

```
# Large MMHW
Length_Stats_LMHW_NO %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  shapiro_test(Growth_Trif)
```

```
## # A tibble: 12 x 6
##   Population Heat_Treatment Phase      variable      statistic      p
##   <fct>      <fct>      <fct>      <chr>          <dbl>  <dbl>
## 1 Cantabria Control      MHW      Growth_Trif    0.885 0.119
## 2 Cantabria Control      Recovery Growth_Trif    0.861 0.0594
## 3 Cantabria MHW2        MHW      Growth_Trif    0.974 0.922
## 4 Cantabria MHW2        Recovery Growth_Trif    0.691 0.000682
## 5 Cantabria MHW4        MHW      Growth_Trif    0.900 0.184
## 6 Cantabria MHW4        Recovery Growth_Trif    0.663 0.000158
## 7 Portugal Control      MHW      Growth_Trif    0.881 0.159
## 8 Portugal Control      Recovery Growth_Trif    0.891 0.204
## 9 Portugal MHW2        MHW      Growth_Trif    0.955 0.748
## 10 Portugal MHW2        Recovery Growth_Trif    0.723 0.00266
## 11 Portugal MHW4        MHW      Growth_Trif    0.861 0.0994
## 12 Portugal MHW4        Recovery Growth_Trif    0.898 0.238
```

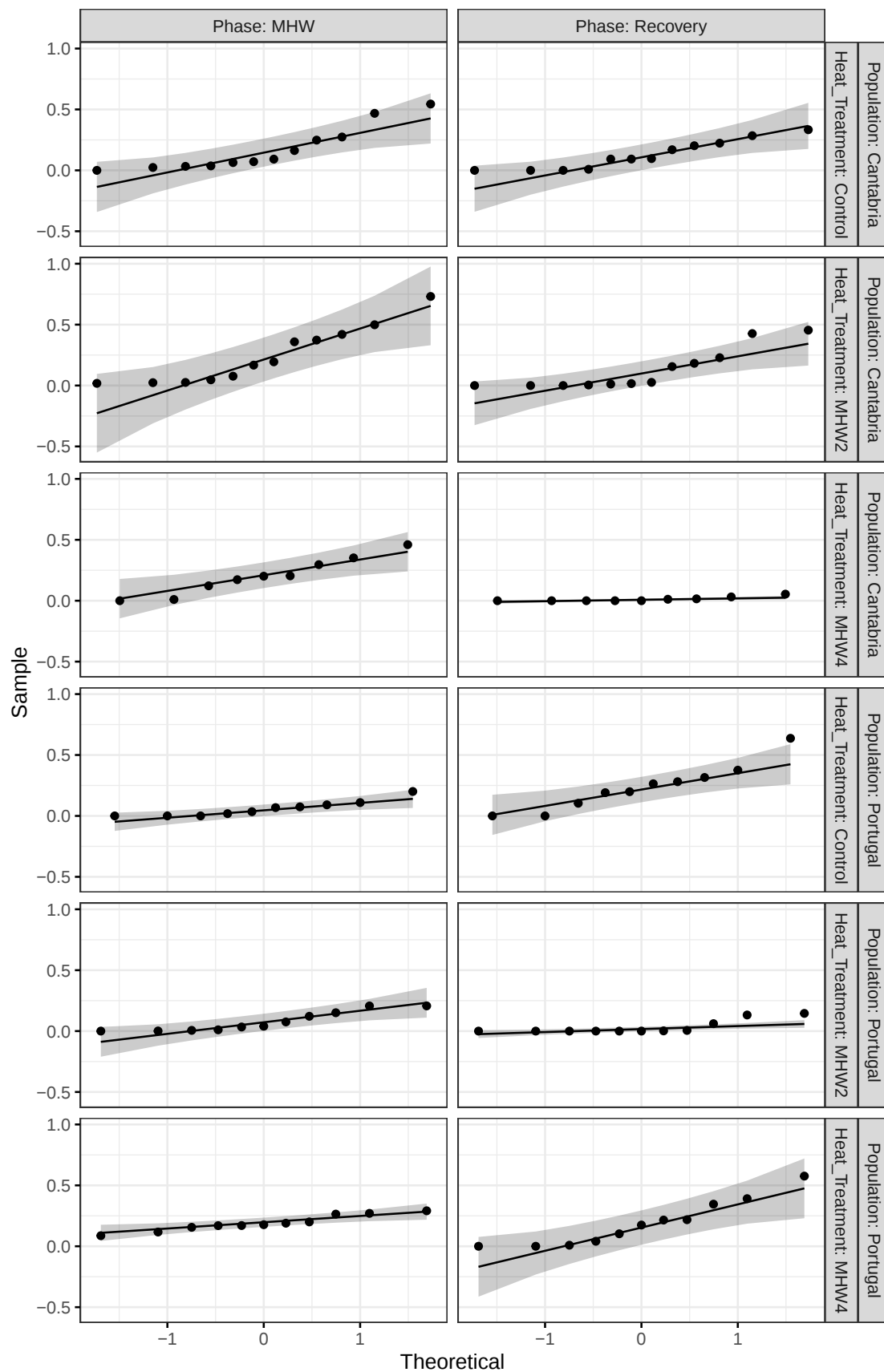
```
ggqqplot(Length_Stats_LMHW_NO, "Growth_Trif", ggtheme = theme_bw()) +
  facet_grid(Population ~ Heat_Treatment ~ Phase, labeller = "label_both")
```



```
# Short MMHW
Length_Stats_SMHW_NO %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  shapiro_test(Growth_Trif)
```

```
## # A tibble: 12 x 6
##   Population Heat_Treatment Phase   variable   statistic      p
##   <fct>      <fct>        <fct>   <chr>      <dbl>    <dbl>
## 1 Cantabria Control      MHW      Growth_Trif 0.852 0.0394
## 2 Cantabria Control      Recovery Growth_Trif 0.903 0.174
## 3 Cantabria MHW2         MHW      Growth_Trif 0.898 0.147
## 4 Cantabria MHW2         Recovery Growth_Trif 0.775 0.00493
## 5 Cantabria MHW4         MHW      Growth_Trif 0.961 0.808
## 6 Cantabria MHW4         Recovery Growth_Trif 0.746 0.00490
## 7 Portugal  Control      MHW      Growth_Trif 0.885 0.147
## 8 Portugal  Control      Recovery Growth_Trif 0.952 0.689
## 9 Portugal  MHW2         MHW      Growth_Trif 0.853 0.0470
## 10 Portugal MHW2         Recovery Growth_Trif 0.624 0.0000508
## 11 Portugal MHW4         MHW      Growth_Trif 0.949 0.633
## 12 Portugal MHW4         Recovery Growth_Trif 0.916 0.289
```

```
ggqqplot(Length_Stats_SMHW_NO, "Growth", ggtheme = theme_bw()) +
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")
```



2.1.3. Homogeneity of variances

Clear heteroscedasticity was observed for both MHW durations. Data transformations (log10 transf.) did not correct it. Finally, according to these results, non-parametric transformations (Aligned Rank Transformation) were used to analyse data.

```
# Large MMHW
Length_Stats_LMHW_NO %>%
  levene_test(Growth_Trif ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4
##   df1 df2 statistic      p
##   <int> <int>     <dbl> <dbl>
## 1    11  106      2.19 0.0201
```

```
Length_Stats_LMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(Growth_Trif ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Population Phase      df1 df2 statistic      p
##   <fct>      <fct>    <int> <int>     <dbl> <dbl>
## 1 Cantabria MHW         2    29      0.707 0.501
## 2 Portugal  MHW         2    24      3.20 0.0585
## 3 Cantabria Recovery     2    29      1.52 0.236
## 4 Portugal  Recovery     2    24     11.8 0.000276
```

```
# Short MMHW
Length_Stats_SMHW_NO %>%
  levene_test(Growth_Trif ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4
##   df1 df2 statistic      p
##   <int> <int>     <dbl> <dbl>
## 1    11  118      2.91 0.00196
```

```
Length_Stats_SMHW_NO %>%
  group_by(Phase) %>%
  levene_test(Growth_Trif ~ Heat_Treatment*Population)
```

```
## # A tibble: 2 x 5
##   Phase      df1 df2 statistic      p
##   <fct>    <int> <int>     <dbl> <dbl>
## 1 MHW         5    59      3.33 0.0102
## 2 Recovery     5    59      3.07 0.0158
```

```
Length_Stats_SMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(Growth_Trif ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Population Phase      df1    df2 statistic      p
##   <fct>      <fct>    <int> <int>    <dbl>   <dbl>
## 1 Cantabria MHW         2     30     0.838 0.442
## 2 Portugal  MHW         2     29     0.661 0.524
## 3 Cantabria Recovery     2     30     3.01 0.0642
## 4 Portugal  Recovery     2     29     4.55 0.0191
```

2.2. Statistical analysis: Mixed model on Aligned Rank Transform (ART)

Repeated measures on the same individuals and the lack of independence among replicates was considered by fitting linear models with random effects (mixed models). For repeated measures, the error associated to each individual (ID) was considered as a random effect while the lack of truly replicates independence was considered by adding Tank as a random factor. Population, MHW intensity and Time were the fixed factors. Then, the structure of the mixed model is as follows: $PAM \sim Population * Heat_Treatment * Phase + (1 | ID) + (1 | Tank)$

From the non-parametric analysis, for the Large MHW we observed that there was not a statistically significant ($p > 0.05$) three-way interaction between time, population, and MHW intensity $F[2, 52] = 1.70$, $p = 0.193$. There was only a significant simple main effect of the Time on the RGR ($F[2, 4] = 11.27$, $p = 0.025$). For the short MHW, no significant three-way interaction was detected ($F[2, 57] = 0.38$, $p = 0.683$), but there were significant two-way interactions between Population/Time ($F[2, 57] = 8.25$, $p = 0.005$) and Population/MHW intensity ($F[2, 58] = 4.45$, $p = 0.015$).

```
# Large MMHW
Length.art_LRec <- art(Growth~ Population*Heat_Treatment*Phase + (1|ID) + (1|Tank),
                      data = Length_Stats_LMHW_NO)
summary(artlm(Length.art_LRec, c("Population:Heat_Treatment:Phase")))
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: .y ~ Population + Heat_Treatment + Phase + (1 | ID) + (1 | Tank) +
##   Population:Heat_Treatment + Population:Phase + Heat_Treatment:Phase +
##   Population:Heat_Treatment:Phase
## Data: df
##
## REML criterion at convergence: 1106
##
## Scaled residuals:
##   Min      1Q  Median      3Q      Max
## -1.6970 -0.7365 -0.1123  0.7295  1.6812
##
## Random effects:
##   Groups   Name                Variance Std.Dev.
##   ID       (Intercept) 1.231e+02 1.110e+01
##   Tank     (Intercept) 3.054e-07 5.526e-04
##   Residual                    1.093e+03 3.306e+01
## Number of obs: 118, groups: ID, 59; Tank, 12
##
## Fixed effects:
##                                     Estimate Std. Error t value
## (Intercept)                        59.7519     3.3827  17.664
## Population1                       -3.7110     3.3827  -1.097
## Heat_Treatment1                   -2.3252     4.7662  -0.488
```

```

## Heat_Treatment2          -1.5492      4.8191  -0.321
## Phase1                   0.1555      3.0560   0.051
## Population1:Heat_Treatment1  3.1934      4.7662   0.670
## Population1:Heat_Treatment2 -2.1418      4.8191  -0.444
## Population1:Phase1        1.4703      3.0560   0.481
## Heat_Treatment1:Phase1     0.8673      4.3058   0.201
## Heat_Treatment2:Phase1     3.4195      4.3536   0.785
## Population1:Heat_Treatment1:Phase1  1.0524      4.3058   0.244
## Population1:Heat_Treatment2:Phase1 -7.3953      4.3536  -1.699
##
## Correlation of Fixed Effects:
##      (Intr) Ppltn1 Ht_Tr1 Ht_Tr2 Phase1 Pp1:H_T1 Pp1:H_T2 Pp1:P1 H_T1:P
## Population1 -0.084
## Het_Trtmnt1 -0.010 -0.010
## Het_Trtmnt2  0.021  0.021 -0.506
## Phase1      0.000  0.000  0.000  0.000
## Ppltn1:H_T1 -0.010 -0.010 -0.092  0.034  0.000
## Ppltn1:H_T2  0.021  0.021  0.034 -0.068  0.000 -0.506
## Ppltn1:P1s1  0.000  0.000  0.000  0.000 -0.084  0.000  0.000
## Ht_Trtn1:P1  0.000  0.000  0.000  0.000 -0.010  0.000  0.000 -0.010
## Ht_Trtn2:P1  0.000  0.000  0.000  0.000  0.021  0.000  0.000  0.021 -0.506
## Pp1:H_T1:P1  0.000  0.000  0.000  0.000 -0.010  0.000  0.000 -0.010 -0.092
## Pp1:H_T2:P1  0.000  0.000  0.000  0.000  0.021  0.000  0.000  0.021  0.034
##      H_T2:P P1:H_T1:
## Population1
## Het_Trtmnt1
## Het_Trtmnt2
## Phase1
## Ppltn1:H_T1
## Ppltn1:H_T2
## Ppltn1:P1s1
## Ht_Trtn1:P1
## Ht_Trtn2:P1
## Pp1:H_T1:P1  0.034
## Pp1:H_T2:P1 -0.068 -0.506
## optimizer (nlptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')

```

```

Length.aov_LRec <- anova(Length.art_LRec)
print(Length.aov_LRec)

```

```

## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Growth)
##
##      F Df  Df.res  Pr(>F)
## 1 Population      0.65782  1 52.4812 0.42099
## 2 Heat_Treatment  2.76576  2  6.9655 0.13058
## 3 Phase          11.27945  1  4.3741 0.02472 *
## 4 Population:Heat_Treatment  0.41779  2 52.4363 0.66068
## 5 Population:Phase    2.74743  1 52.1775 0.10341
## 6 Heat_Treatment:Phase  2.48593  2  4.4109 0.18925

```

```
## 7 Population:Heat_Treatment:Phase 1.68743 2 52.1422 0.19494
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Short MMHW
```

```
Length.art_SRec <- art(Growth ~ Population*Heat_Treatment*Phase + (1|ID) + (1|Tank),
  data = Length_Stats_SMHW_NO)
summary(artlm(Length.art_SRec, c("Population:Heat_Treatment:Phase")))
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: .y ~ Population + Heat_Treatment + Phase + (1 | ID) + (1 | Tank) +
##      Population:Heat_Treatment + Population:Phase + Heat_Treatment:Phase +
##      Population:Heat_Treatment:Phase
##      Data: df
##
## REML criterion at convergence: 1251.2
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.6473 -0.6896 -0.0456  0.7077  1.7349
##
## Random effects:
##      Groups      Name                Variance Std.Dev.
##      ID          (Intercept)         263.6    16.24
##      Tank        (Intercept)           0.0     0.00
##      Residual                        1251.9    35.38
## Number of obs: 130, groups:  ID, 65; Tank, 12
##
## Fixed effects:
##
##              Estimate Std. Error t value
## (Intercept)      65.8407     3.7187  17.705
## Population1      -2.0259     3.7187  -0.545
## Heat_Treatment1  -1.5616     5.2364  -0.298
## Heat_Treatment2  -1.7593     5.1716  -0.340
## Phase1           0.3850     3.1193   0.123
## Population1:Heat_Treatment1 -0.7949     5.2364  -0.152
## Population1:Heat_Treatment2 -2.8472     5.1716  -0.551
## Population1:Phase1 -1.5332     3.1193  -0.492
## Heat_Treatment1:Phase1 -3.1725     4.3925  -0.722
## Heat_Treatment2:Phase1  1.0525     4.3382   0.243
## Population1:Heat_Treatment1:Phase1  2.9457     4.3925   0.671
## Population1:Heat_Treatment2:Phase1 -3.5293     4.3382  -0.814
##
## Correlation of Fixed Effects:
##              (Intr) Ppltn1 Ht_Tr1 Ht_Tr2 Phase1 Pp1:H_T1 Pp1:H_T2 Pp1:P1 H_T1:P
## Population1 -0.007
## Het_Trmtnt1 -0.012 -0.058
## Het_Trmtnt2 -0.047 -0.024 -0.468
## Phase1      0.000  0.000  0.000  0.000
## Ppltn1:H_T1 -0.058 -0.012 -0.049  0.063  0.000
## Ppltn1:H_T2 -0.024 -0.047  0.063 -0.025  0.000 -0.468
## Ppltn1:P1s1  0.000  0.000  0.000  0.000 -0.007  0.000  0.000
## Ht_Trmt1:P1  0.000  0.000  0.000  0.000 -0.012  0.000  0.000 -0.058
## Ht_Trmt2:P1  0.000  0.000  0.000  0.000 -0.047  0.000  0.000 -0.024 -0.468
```

```
## Pp1:H_T1:P1 0.000 0.000 0.000 0.000 -0.058 0.000 0.000 -0.012 -0.049
## Pp1:H_T2:P1 0.000 0.000 0.000 0.000 -0.024 0.000 0.000 -0.047 0.063
## H_T2:P P1:H_T1:
## Population1
## Het_Trtmnt1
## Het_Trtmnt2
## Phase1
## Ppltn1:H_T1
## Ppltn1:H_T2
## Ppltn1:Phs1
## Ht_Trtm1:P1
## Ht_Trtm2:P1
## Pp1:H_T1:P1 0.063
## Pp1:H_T2:P1 -0.025 -0.468
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
Length.aov_SRec <- anova(Length.art_SRec)
print(Length.aov_SRec)
```

```
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Growth)
##
##
```

	F	Df	Df.res	Pr(>F)
## 1 Population	0.047315	1	58.2209	0.828563
## 2 Heat_Treatment	0.996007	2	7.9241	0.411273
## 3 Phase	2.956026	1	4.3604	0.154734
## 4 Population:Heat_Treatment	4.451001	2	57.9436	0.015915 *
## 5 Population:Phase	8.256180	1	57.5145	0.005681 **
## 6 Heat_Treatment:Phase	5.314643	2	4.6198	0.063392 .
## 7 Population:Heat_Treatment:Phase	0.382616	2	57.4956	0.683798

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.3. Post-hoc analysis

For the Large MHW:

Not needed as there was only a significant simple effect of Time.

For the Short MHW:

1. Simple two-way interaction analysis: It was not computed considering that there was not significant three-way interactions among treatments.
2. Simple-simple main effect analysis: There was not any statistically significant simple simple main effect.
3. Simple comparisons: No significant differences.

```

# Short MHW
MHW.effect_SRec <- list()
i=1
j=1
for (i in 1:2) {
  a <- Length_Stats_SMHW_NO[Length_Stats_SMHW_NO$Phase == as.character(
    unique(Length_Stats_SMHW_NO$Phase)[i]),]
  temp <- list()
  for (j in 1:2) {
    b <- a[a$Population == as.character(unique(a$Population)[j]),]
    c <- art(Growth ~ Heat_Treatment + (1|Tank) , data = b)
    d <- anova(c)
    temp[[j]] <- d
    names(temp)[[j]] <- paste(as.character(unique(Length_Stats_SMHW_NO$Phase)[i]),
      "_",as.character(unique(a$Population)[j]))
    rm(b,c,d)
  }
  MHW.effect_SRec[[i]] <- temp
  rm(temp)
}
MHW.effect_SRec

## [[1]]
## [[1]]$'MHW _ Cantabria'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Growth)
##
##              F Df Df.res  Pr(>F)
## 1 Heat_Treatment 0.30787  2 3.0377 0.75553
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## [[1]]$'MHW _ Portugal'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Growth)
##
##              F Df Df.res  Pr(>F)
## 1 Heat_Treatment 3.4679  2 2.9784 0.16682
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## [[2]]
## [[2]]$'Recovery _ Cantabria'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)

```

```
## Model: Mixed Effects (lmer)
## Response: art(Growth)
##
##               F Df Df.res  Pr(>F)
## 1 Heat_Treatment 2.6571  2 3.0377 0.21521
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## [[2]]$'Recovery _ Portugal'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Growth)
##
##               F Df Df.res  Pr(>F)
## 1 Heat_Treatment 4.8722  2 2.8613 0.11986
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pwc_SRec <- list()
i=1
j=1
for (i in 1:2) {
  a <- Length_Stats_SMHW_NO[Length_Stats_SMHW_NO$Phase == as.character(
    unique(Length_Stats_SMHW_NO$Phase)[i]),]
  temp <- list()
  for (j in 1:2) {
    b <- a[a$Population == as.character(unique(a$Population)[j]),]
    c <- art(Growth~ Heat_Treatment + (1|Tank), data = b)
    d <- art.con(c, "Heat_Treatment")
    d <- as.data.frame(d)
    d$Phase <- as.character(unique(Length_Stats_SMHW_NO$Phase)[i])
    d$Population <- as.character(unique(a$Population)[j])
    temp[[j]] <- d
    rm(b,c,d)
  }
  temp <- do.call(rbind, temp)
  pwc_SRec[[i]] <- temp
  rm(temp)
}

pwc_SRec <- as.data.frame(do.call(rbind, pwc_SRec))
pwc_SRec$Duration_Treatment <- "S.Rec"
pwc_SRec
```

## contrast	estimate	SE	df	t.ratio	p.value	Phase	Population
## Control - MHW2	-3.125000	4.035285	2.55	-0.774	0.7457	MHW	Cantabria
## Control - MHW4	-2.097222	4.373446	3.42	-0.480	0.8850	MHW	Cantabria
## MHW2 - MHW4	1.027778	4.373446	3.42	0.235	0.9703	MHW	Cantabria
## Control - MHW2	-2.034780	5.374941	3.01	-0.379	0.9259	MHW	Portugal
## Control - MHW4	-13.084729	5.374941	3.01	-2.434	0.1771	MHW	Portugal
## MHW2 - MHW4	-11.049949	5.318290	2.91	-2.078	0.2452	MHW	Portugal
## Control - MHW2	1.250000	3.684029	2.55	0.339	0.9400	Recovery	Cantabria

```

## Control - MHW4      8.722222 3.992754 3.42    2.185  0.2044 Recovery Cantabria
## MHW2 - MHW4        7.472222 3.992754 3.42    1.871  0.2758 Recovery Cantabria
## Control - MHW2     11.229102 3.883113 2.92    2.892  0.1256 Recovery Portugal
## Control - MHW4      2.042444 3.883113 2.92    0.526  0.8651 Recovery Portugal
## MHW2 - MHW4        -9.186658 3.774491 2.75   -2.434  0.1890 Recovery Portugal
## Duration_Treatment
## S.Rec
## S.Rec
## S.Rec
## S.Rec
## S.Rec
## S.Rec
## S.Rec
## S.Rec
## S.Rec
## S.Rec
## S.Rec
## S.Rec
## S.Rec
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates

```

Chlorophyll-a fluorescence annalysis: Maximum quantum efficiency of primary photochemistry of photosystem II (Fv/Fm)

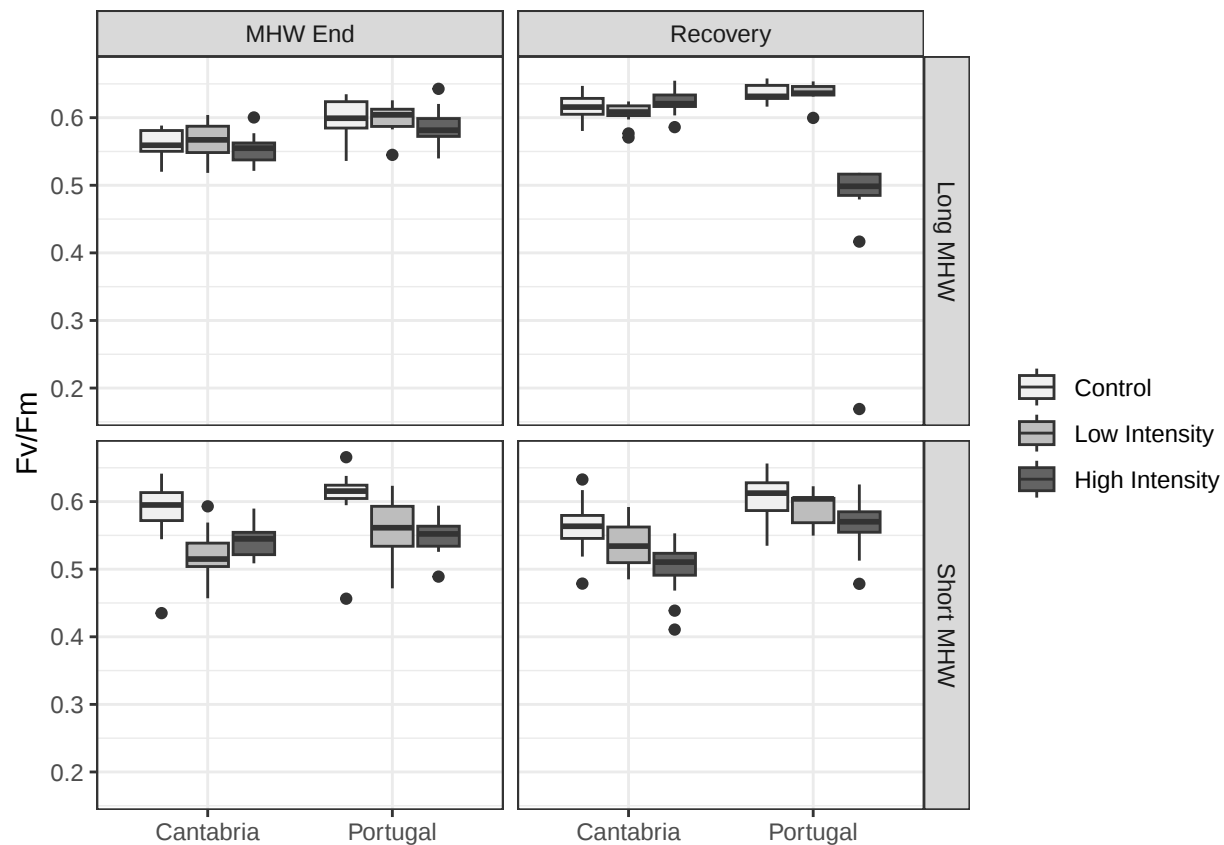
1. DATA PROCESSING

Data loading and inspection. First of all, we will visualize raw data using a boxplot.

```
# Data processing
PAM <- data.frame(read.xlsx("D:/Estancia_Porto_2023/experiments/MHW/Full_Datasets.xlsx",
                           sheet = 7))
PAM <- PAM[PAM$Duration_Treatment == "Srec" | PAM$Duration_Treatment == "Lrec",]
names(PAM)[10] <- "PAM"
PAM <- na.omit(PAM)

# Labels
day.labs <- c("MHW End", "Recovery")
names(day.labs) <- c("MHW", "Recovery")
Duration.labs <- c("Short MHW", "Long MHW")
names(Duration.labs) <- c("Srec", "Lrec")
MHW.labs <- c("Control", "Low Intensity", "High Intensity")

# Plot
PAM_plot <- ggplot(PAM, aes(x = as.factor(Population), y = PAM,
                           fill = as.factor(Heat_Treatment))) +
  geom_boxplot() +
  facet_grid(Duration_Treatment~Phase,
             labeller=labeler(Duration_Treatment=Duration.labs,Phase=day.labs)) +
  labs(x = NULL, y = 'Fv/Fm', fill = NULL) +
  theme(text=element_text(family=windowsFont("Arial")),
        axis.text.x = element_text(color = "grey20", size = 14),
        axis.text.y = element_text(color = "grey20", size = 14),
        axis.title.x = element_text(color = "grey20", size = 16),
        axis.title.y = element_text(color = "grey20", size = 16),
        strip.text.x = element_text(color = "grey20", size = 16),
        strip.text.y = element_text(color = "grey20", size = 16),
        legend.text = element_text(color = "grey20", size = 14),
        legend.background = element_rect(colour = "grey20", fill = "white", size = 0.3)) +
  scale_fill_brewer(palette="Greys", labels = MHW.labs) + theme_bw()
PAM_plot
```



```
# Data preparation for statistical analysis
```

```
PAM_Stats <- PAM
PAM_Stats$ID <- as.factor(PAM_Stats$ID)
PAM_Stats$Phase <- as.factor(PAM_Stats$Phase)
PAM_Stats$Heat_Treatment <- as.factor(PAM_Stats$Heat_Treatment)
PAM_Stats$Population <- as.factor(PAM_Stats$Population)
PAM_Stats$Tank <- as.factor(PAM_Stats$Tank)
summary(PAM_Stats)
```

```
##      Day      Population Heat_Treatment Duration_Treatment
## Min.   : 7.00   Cantabria:144   Control:96   Length:288
## 1st Qu.:13.00   Portugal :144   MHW2 :96     Class :character
## Median :18.00                                MHW4 :96     Mode  :character
## Mean   :17.75
## 3rd Qu.:22.75
## Max.   :28.00
##
##      Phase      Replicate      Plant      ID      Tank
## MHW      :144   Length:288   Min.    :1.0   1      : 4   1      : 24
## Recovery:144   Class :character 1st Qu.:2.0   2      : 4   2      : 24
##                      Mode  :character Median :3.5   3      : 4   3      : 24
##                      Mean   :3.5   4      : 4   4      : 24
##                      3rd Qu.:5.0   5      : 4   5      : 24
##                      Max.   :6.0   6      : 4   6      : 24
##                      (Other):264 (Other):144
##      PAM
```

```
## Min.      :0.1690
## 1st Qu.:0.5443
## Median :0.5832
## Mean      :0.5740
## 3rd Qu.:0.6157
## Max.      :0.6657
##
```

```
PAM_Stats_SMHW <- PAM_Stats[PAM_Stats$Duration_Treatment == "Srec",]
PAM_Stats_LMHW <- PAM_Stats[PAM_Stats$Duration_Treatment == "Lrec",]
```

2. STATISTICAL ANALYSIS

To test the effects of the different treatments (Population, Low/High Intensity MHW) on the Maximum quantum efficiency of primary photochemistry of photosystem II (Fv/Fm) we follow a factorial design with repeated measures. The analysis was performed separately for each MHW (Long/Short) as the results were compared to independent control treatments. In this context, **POPULATION** (w/ two levels: Cantabrian and Atlantic) and **MHW INTENSITY** (w/ three levels: Control, Low Intensity and High intensity) were considered between-subject factors (fixed), while **TIME** (w/ two levels: after MHW and after recovery period) was considered a within-subject factor. The response variable measured was the **Fv/Fm**. The aim was to understand how Fv/Fm values change over time (After Stress Period vs Recovery) depending on the population of origin and the induced thermal stress imposed by the MHW (Low vs High vs Non-Stress).

2.1. Check Model assumptions

2.1.1. Outliers

First of all, the outliers were identified and removed when considered extreme.

```
# Large MHW
outliers_LMHW <- PAM_Stats_LMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(PAM)
outliers_LMHW <- outliers_LMHW[-c(11,12)]
PAM_Stats_LMHW_NO <- anti_join(PAM_Stats_LMHW, outliers_LMHW, by = c("ID"))
outliers_LMHW %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, PAM) %>%
  head() # Large MHW outliers
```

```
## # A tibble: 6 x 7
##   Phase      Population Heat_Treatment    Day Duration_Treatment ID      PAM
##   <fct>      <fct>      <fct>      <dbl> <chr>      <fct> <dbl>
## 1 Recovery Cantabria MHW2         28 Lrec       34    0.577
## 2 Recovery Cantabria MHW2         28 Lrec       35    0.571
## 3 MHW       Cantabria MHW4         21 Lrec       11    0.600
## 4 Recovery Cantabria MHW4         28 Lrec       11    0.586
## 5 MHW       Portugal  MHW2         21 Lrec       46    0.545
## 6 Recovery Portugal  MHW2         28 Lrec       39    0.600
```

```
# Short MHW
outliers_SMHW <- PAM_Stats_SMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(PAM)
outliers_SMHW <- outliers_SMHW[-c(11,12)]
PAM_Stats_SMHW_NO <- anti_join(PAM_Stats_SMHW, outliers_SMHW, by = c("ID"))
outliers_SMHW %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, PAM) %>%
  head() # Short MHW outliers
```

```
## # A tibble: 6 x 7
##   Phase      Population Heat_Treatment   Day Duration_Treatment ID      PAM
##   <fct>      <fct>      <fct>      <dbl> <chr>                <fct> <dbl>
## 1 MHW        Cantabria   Control      7 Srec                49    0.435
## 2 Recovery  Cantabria   Control     15 Srec                49    0.479
## 3 Recovery  Cantabria   Control     15 Srec                51    0.633
## 4 MHW        Cantabria   MHW2        7 Srec                31    0.593
## 5 Recovery  Cantabria   MHW4       15 Srec                11    0.411
## 6 Recovery  Cantabria   MHW4       15 Srec                12    0.439
```

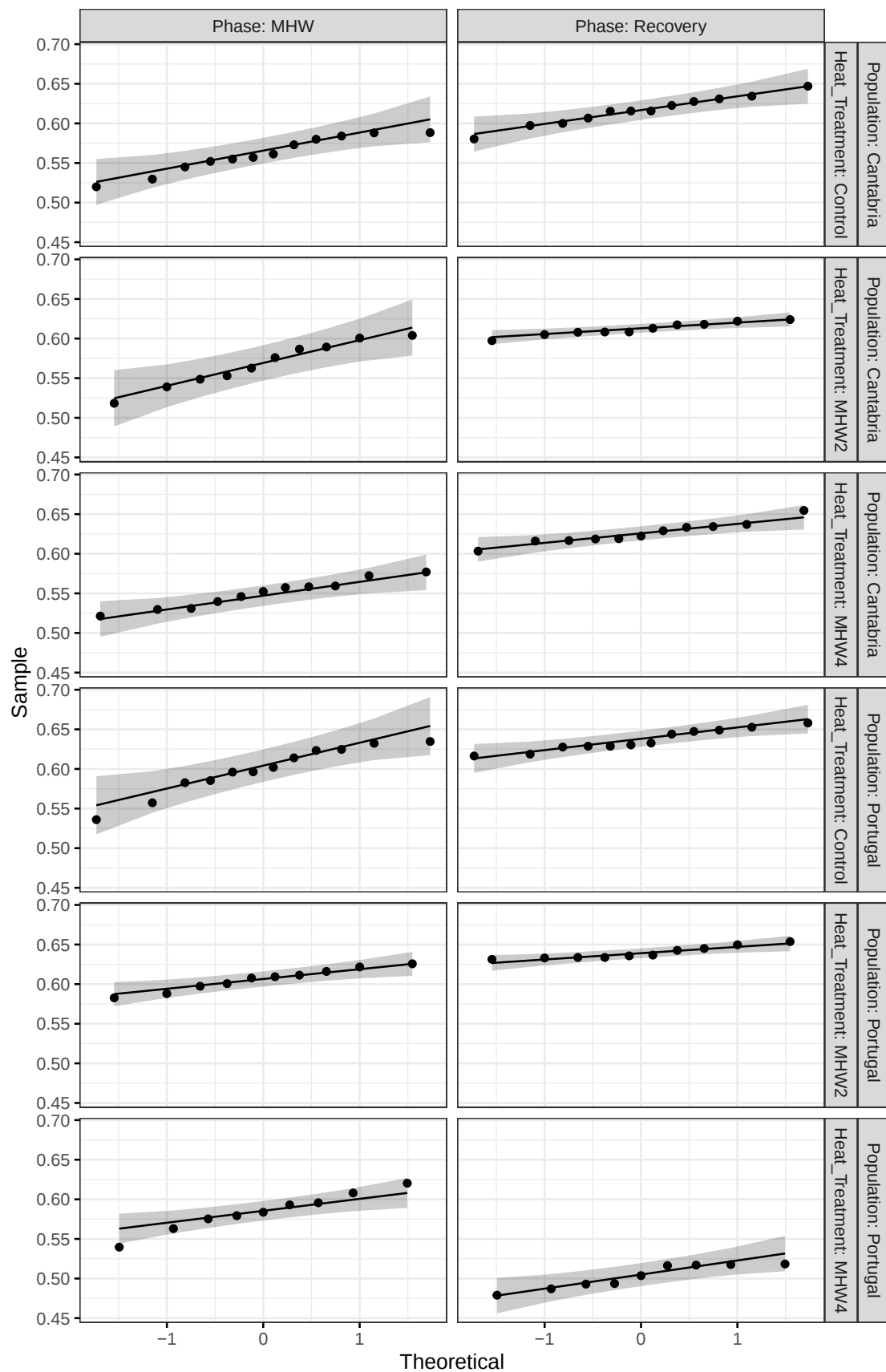
2.1.2. Normality

The second step was to test normality. For that purpose, we computed Shapiro-Wilk's tests for each combinations of factor levels. Normality was also assessed visually by inspecting qqplots. According to the results, no clear deviations from normality were observed (neither from tests nor from the qqplots).

```
# Large MMHW
PAM_Stats_LMHW_NO %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  shapiro_test(PAM)
```

```
## # A tibble: 12 x 6
##   Population Heat_Treatment Phase   variable statistic      p
##   <fct>      <fct>      <fct>   <chr>      <dbl> <dbl>
## 1 Cantabria Control      MHW      PAM        0.937 0.463
## 2 Cantabria Control      Recovery PAM        0.985 0.996
## 3 Cantabria MHW2        MHW      PAM        0.957 0.754
## 4 Cantabria MHW2        Recovery PAM        0.960 0.790
## 5 Cantabria MHW4        MHW      PAM        0.964 0.820
## 6 Cantabria MHW4        Recovery PAM        0.955 0.706
## 7 Portugal  Control      MHW      PAM        0.931 0.389
## 8 Portugal  Control      Recovery PAM        0.935 0.438
## 9 Portugal  MHW2        MHW      PAM        0.968 0.874
## 10 Portugal MHW2        Recovery PAM        0.879 0.128
## 11 Portugal MHW4        MHW      PAM        0.984 0.983
## 12 Portugal MHW4        Recovery PAM        0.868 0.118
```

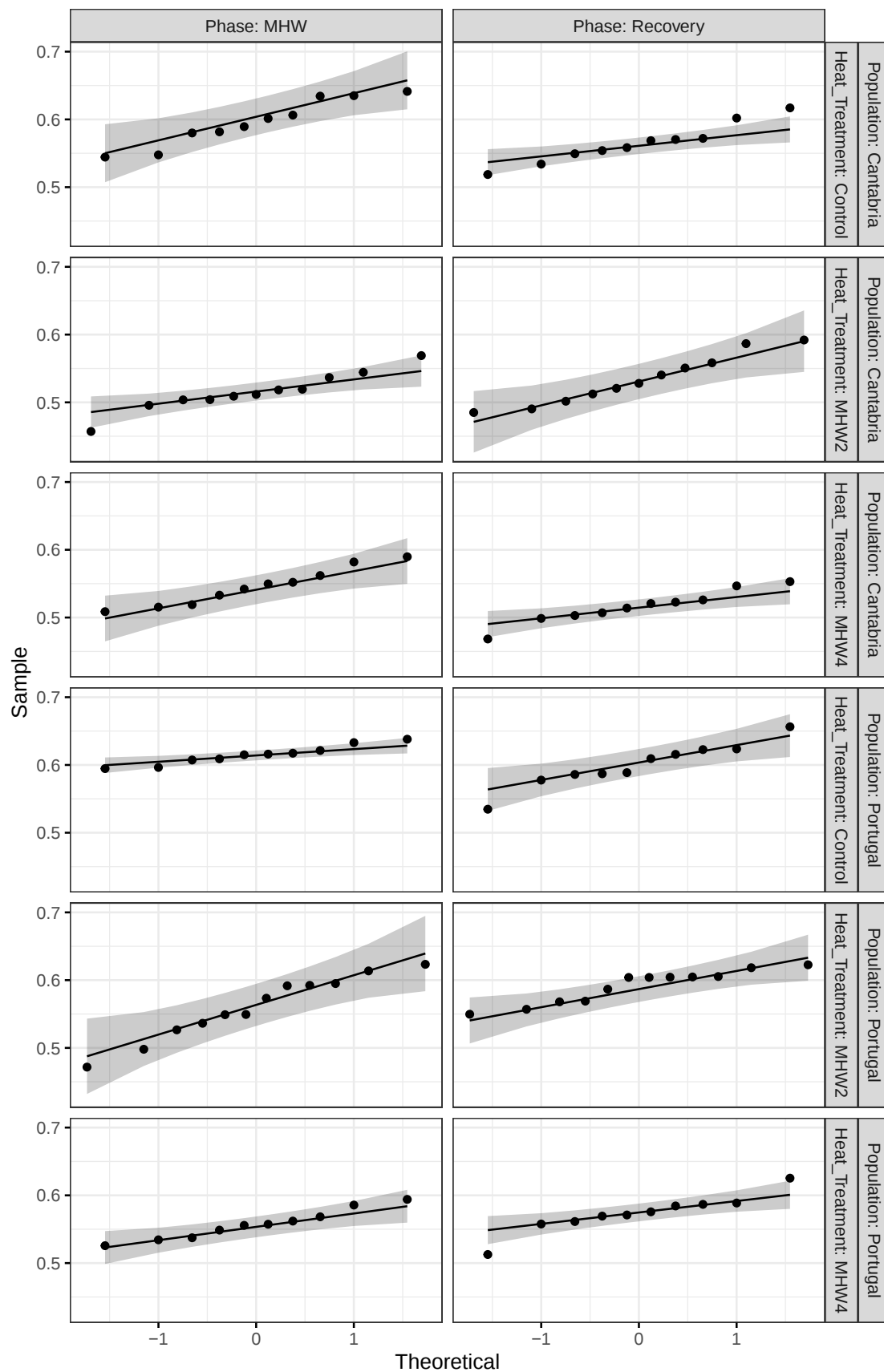
```
ggqqplot(PAM_Stats_LMHW_NO, "PAM", ggtheme = theme_bw()) +
  facet_grid(Population ~ Heat_Treatment ~ Phase, labeller = "label_both")
```



```
# Short MMHW
PAM_Stats_SMHW_NO %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  shapiro_test(PAM)
```

```
## # A tibble: 12 x 6
##   Population Heat_Treatment Phase   variable statistic     p
##   <fct>      <fct>          <fct>   <chr>      <dbl> <dbl>
## 1 Cantabria Control       MHW     PAM        0.927 0.416
## 2 Cantabria Control       Recovery PAM        0.965 0.836
## 3 Cantabria MHW2         MHW     PAM        0.957 0.731
## 4 Cantabria MHW2         Recovery PAM        0.951 0.658
## 5 Cantabria MHW4         MHW     PAM        0.954 0.716
## 6 Cantabria MHW4         Recovery PAM        0.964 0.828
## 7 Portugal  Control       MHW     PAM        0.957 0.749
## 8 Portugal  Control       Recovery PAM        0.959 0.774
## 9 Portugal  MHW2         MHW     PAM        0.955 0.716
## 10 Portugal MHW2         Recovery PAM        0.891 0.121
## 11 Portugal MHW4         MHW     PAM        0.966 0.853
## 12 Portugal MHW4         Recovery PAM        0.923 0.386
```

```
ggqqplot(PAM_Stats_SMHW_NO, "PAM", ggtheme = theme_bw()) +
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")
```



2.1.3. Homogeneity of variances

Homogeneity of variances was checked through Levene's tests. For the large MHW, tests using the complete dataset revealed heteroscedasticity. A deeper analysis, grouping data per Phase, showed that heterogeneity of variances was mainly observed for the Recovery, as a consequence of the increased deviance registered for the Atlantic population cultured at the high intensity MHW (leading to a $p < 0.01$). This is congruent with the existing knowledge on how Fv/Fm may respond. When the photosynthetic apparatus is not stressed or when the stress is weak, the Fv/Fm values are generally high (~ 0.6), while increasing levels of stress tend to reduce that value and make it more inconsistent as the different parts of the thallus may respond differently (leading to that increased variance). Even though homogeneity was not fully achieved, it was decided to continue with the analysis and try to reduce the effect by applying a heteroscedasticity correction to the coefficient of covariance matrix. Results for that phase will be interpreted more carefully. For the short MHW, variances were homogeneous ($p > 0.05$), so final conclusions would be stronger.

```
# Large MMHW
PAM_Stats_LMHW_NO %>%
  levene_test(PAM ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4
##   df1    df2 statistic      p
##   <int> <int>     <dbl>  <dbl>
## 1     11    116      2.74 0.00348
```

```
PAM_Stats_LMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(PAM ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Population Phase      df1    df2 statistic      p
##   <fct>      <fct>    <int> <int>     <dbl>  <dbl>
## 1 Cantabria  MHW         2     30      1.54 0.231
## 2 Portugal   MHW         2     28      1.92 0.166
## 3 Cantabria  Recovery     2     30      1.55 0.228
## 4 Portugal   Recovery     2     28      2.44 0.105
```

```
# Short MMHW
PAM_Stats_SMHW_NO %>%
  levene_test(PAM ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4
##   df1    df2 statistic      p
##   <int> <int>     <dbl>  <dbl>
## 1     11    114      1.65 0.0937
```

```
PAM_Stats_SMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(PAM ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Population Phase      df1    df2 statistic      p
##   <fct>      <fct>    <int> <int>     <dbl>  <dbl>
```

## 1	Cantabria	MHW	2	28	0.499	0.612
## 2	Portugal	MHW	2	29	8.02	0.00169
## 3	Cantabria	Recovery	2	28	1.01	0.378
## 4	Portugal	Recovery	2	29	0.373	0.692

2.2. Statistical analysis: Mixed model

Repeated measures on the same individuals and the lack of independence among replicates was considered by fitting linear models with random effects (mixed models). For repeated measures, the error associated to each individual (ID) was considered as a random effect while the lack of truly replicates independence was considered by adding Tank as a random factor. Population, MHW intensity and Time were the fixed factors. Then, the structure of the mixed model is as follows: $PAM \sim Population * Heat_Treatment * Phase + (1 | Tank) + (1 | Tank:ID)$

From the model for the Large MHW what we observed was that there was a statistically significant ($p < .05$) three-way interaction between time, population, and MHW intensity $F[2, 58] = 56.28$, $p < 0.0001$. For the short MHW, no significant three-way interaction was detected ($F[2, 57] = 1.06$, $p = 0.353$), but there were significant two-way interactions between Population/Time ($F[1, 57] = 6.540$, $p = 0.013$) and Time/MHW intensity ($F[2, 57] = 8.261$, $p < 0.001$).

```
#LRec
lmer_LRec_2 <- lmerTest::lmer(PAM ~ Population*Heat_Treatment*Phase
                             + (1 | ID) + (1 | Tank), #Rep. meas. + random tank effects
                             PAM_Stats_LMHW_NO, REML = TRUE)

summary(lmer_LRec_2, ddf="Kenward-Roger") #LRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: PAM ~ Population * Heat_Treatment * Phase + (1 | ID) + (1 | Tank)
## Data: PAM_Stats_LMHW_NO
##
## REML criterion at convergence: -560.6
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.00529 -0.52840  0.04574  0.62349  1.88985
##
## Random effects:
## Groups Name Variance Std.Dev.
## ID      (Intercept) 0.0000581 0.007622
## Tank     (Intercept) 0.0000000 0.000000
## Residual                    0.0003116 0.017652
## Number of obs: 128, groups: ID, 64; Tank, 6
##
## Fixed effects:
##
##              Estimate Std. Error
## (Intercept)    5.611e-01  5.550e-03
## PopulationPortugal    3.761e-02  7.850e-03
## Heat_TreatmentMHW2    6.722e-03  8.264e-03
## Heat_TreatmentMHW4   -1.163e-02  8.033e-03
## PhaseRecovery    5.503e-02  7.206e-03
## PopulationPortugal:Heat_TreatmentMHW2    5.889e-04  1.167e-02
```

```

## PopulationPortugal:Heat_TreatmentMHW4 -2.874e-03 1.167e-02
## PopulationPortugal:PhaseRecovery -1.758e-02 1.019e-02
## Heat_TreatmentMHW2:PhaseRecovery -1.073e-02 1.069e-02
## Heat_TreatmentMHW4:PhaseRecovery 2.134e-02 1.042e-02
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 6.750e-03 1.512e-02
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery -1.402e-01 1.516e-02
## df t value Pr(>|t|)
## (Intercept) 2.508e+01 101.093 < 2e-16
## PopulationPortugal 1.093e+02 4.792 5.24e-06
## Heat_TreatmentMHW2 2.848e+01 0.813 0.4227
## Heat_TreatmentMHW4 2.686e+01 -1.447 0.1594
## PhaseRecovery 5.800e+01 7.636 2.50e-10
## PopulationPortugal:Heat_TreatmentMHW2 1.101e+02 0.050 0.9598
## PopulationPortugal:Heat_TreatmentMHW4 1.094e+02 -0.246 0.8060
## PopulationPortugal:PhaseRecovery 5.800e+01 -1.725 0.0898
## Heat_TreatmentMHW2:PhaseRecovery 5.800e+01 -1.004 0.3197
## Heat_TreatmentMHW4:PhaseRecovery 5.800e+01 2.048 0.0451
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 5.800e+01 0.447 0.6569
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 5.800e+01 -9.246 5.22e-13
##
## (Intercept) ***
## PopulationPortugal ***
## Heat_TreatmentMHW2
## Heat_TreatmentMHW4
## PhaseRecovery ***
## PopulationPortugal:Heat_TreatmentMHW2
## PopulationPortugal:Heat_TreatmentMHW4
## PopulationPortugal:PhaseRecovery .
## Heat_TreatmentMHW2:PhaseRecovery
## Heat_TreatmentMHW4:PhaseRecovery *
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
## (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PopltnPrtgl -0.707
## Ht_TrtmMHW2 -0.674 0.477
## Ht_TrtmMHW4 -0.692 0.489 0.466
## PhaseRecvry -0.649 0.459 0.438 0.449
## PpP:H_TMHW2 0.477 -0.674 -0.707 -0.330 -0.309
## PpP:H_TMHW4 0.475 -0.672 -0.321 -0.687 -0.309 0.453
## PpltnPrt:PR 0.459 -0.649 -0.309 -0.317 -0.707 0.438 0.436
## Ht_TMHW2:PR 0.438 -0.309 -0.649 -0.303 -0.674 0.459 0.208
## Ht_TMHW4:PR 0.449 -0.317 -0.303 -0.649 -0.692 0.214 0.446
## PP:H_TMHW2: -0.309 0.438 0.459 0.214 0.477 -0.649 -0.294
## PP:H_TMHW4: -0.309 0.436 0.208 0.446 0.475 -0.294 -0.649
## PpP:PR H_TMHW2: H_TMHW4: PP:H_TMHW2:
## PopltnPrtgl
## Ht_TrtmMHW2
## Ht_TrtmMHW4
## PhaseRecvry
## PpP:H_TMHW2

```

```
## PpP:H_TMHw4
## PpltnPrt:PR
## Ht_TMHw2:PR 0.477
## Ht_TMHw4:PR 0.489 0.466
## PP:H_TMHw2: -0.674 -0.707 -0.330
## PP:H_TMHw4: -0.672 -0.321 -0.687 0.453
```

```
anova(lmer_LRec_2, type = 3, ddf="Kenward-Roger") #LRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##
##              Sum Sq   Mean Sq NumDF  DenDF F value
## Population      0.000779 0.0007787    1 55.384  2.4991
## Heat_Treatment  0.030033 0.0150165    2  2.968 48.1064
## Phase           0.024004 0.0240041    1 58.000 77.0369
## Population:Heat_Treatment 0.027791 0.0138956    2 55.370 44.5951
## Population:Phase 0.030467 0.0304667    1 58.000 97.7775
## Heat_Treatment:Phase 0.014366 0.0071830    2 58.000 23.0525
## Population:Heat_Treatment:Phase 0.035073 0.0175367    2 58.000 56.2808
##
##              Pr(>F)
## Population      0.119606
## Heat_Treatment  0.005476 **
## Phase           3.099e-12 ***
## Population:Heat_Treatment 2.895e-12 ***
## Population:Phase 4.700e-14 ***
## Heat_Treatment:Phase 4.293e-08 ***
## Population:Heat_Treatment:Phase 2.599e-14 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#SRec
lmer_SRec_2 <- lmerTest::lmer(PAM ~ Population*Heat_Treatment*Phase
                             + (1 | ID) + (1 | Tank), #Rep. meas. + random tank effects
                             PAM_Stats_SMHW_NO, REML = TRUE)

summary(lmer_SRec_2, ddf="Kenward-Roger") #SRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: PAM ~ Population * Heat_Treatment * Phase + (1 | ID) + (1 | Tank)
## Data: PAM_Stats_SMHW_NO
##
## REML criterion at convergence: -446.3
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.79242 -0.50208 -0.00609  0.52709  1.78658
##
## Random effects:
## Groups   Name                Variance Std.Dev.
## ID      (Intercept) 0.0001576 0.01256
## Tank    (Intercept) 0.0000000 0.00000
```

```

## Residual          0.0007677 0.02771
## Number of obs: 126, groups: ID, 63; Tank, 6
##
## Fixed effects:
##
##                                     Estimate Std. Error
## (Intercept)                        0.596133   0.009691
## PopulationPortugal                  0.018667   0.013655
## Heat_TreatmentMHW2                 -0.080800   0.013356
## Heat_TreatmentMHW4                 -0.050800   0.013706
## PhaseRecovery                     -0.031700   0.012391
## PopulationPortugal:Heat_TreatmentMHW2  0.026028   0.018655
## PopulationPortugal:Heat_TreatmentMHW4 -0.007100   0.019311
## PopulationPortugal:PhaseRecovery      0.017067   0.017524
## Heat_TreatmentMHW2:PhaseRecovery      0.049639   0.017121
## Heat_TreatmentMHW4:PhaseRecovery      0.002367   0.017524
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery -0.003895   0.023971
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery  0.028633   0.024783
##
##                                     df t value Pr(>|t|)
## (Intercept)                        30.077068  61.512 < 2e-16
## PopulationPortugal                 108.135869   1.367 0.174451
## Heat_TreatmentMHW2                 28.579582  -6.050 1.47e-06
## Heat_TreatmentMHW4                 30.077068  -3.707 0.000847
## PhaseRecovery                     57.000000  -2.558 0.013202
## PopulationPortugal:Heat_TreatmentMHW2 107.708114   1.395 0.165831
## PopulationPortugal:Heat_TreatmentMHW4 108.135869  -0.368 0.713840
## PopulationPortugal:PhaseRecovery     57.000000   0.974 0.334219
## Heat_TreatmentMHW2:PhaseRecovery     57.000000   2.899 0.005302
## Heat_TreatmentMHW4:PhaseRecovery     57.000000   0.135 0.893046
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 57.000000  -0.162 0.871500
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 57.000000   1.155 0.252755
##
## (Intercept)                        ***
## PopulationPortugal
## Heat_TreatmentMHW2                  ***
## Heat_TreatmentMHW4                  ***
## PhaseRecovery                       *
## PopulationPortugal:Heat_TreatmentMHW2
## PopulationPortugal:Heat_TreatmentMHW4
## PopulationPortugal:PhaseRecovery
## Heat_TreatmentMHW2:PhaseRecovery    **
## Heat_TreatmentMHW4:PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PopltnPrtgl -0.707
## Ht_TrtmMHW2 -0.724  0.512
## Ht_TrtmMHW4 -0.707  0.500  0.512
## PhaseRecvry -0.644  0.455  0.466   0.455
## PpP:H_TMHW2  0.517 -0.731 -0.714  -0.366  -0.333
## PpP:H_TMHW4  0.500 -0.707 -0.362  -0.707  -0.322  0.517

```

```
## PpltnPrt:PR 0.455 -0.644 -0.330 -0.322 -0.707 0.471 0.455
## Ht_TMHW2:PR 0.466 -0.330 -0.644 -0.330 -0.724 0.460 0.233
## Ht_TMHW4:PR 0.455 -0.322 -0.330 -0.644 -0.707 0.235 0.455
## PP:H_TMHW2: -0.333 0.471 0.460 0.235 0.517 -0.644 -0.333
## PP:H_TMHW4: -0.322 0.455 0.233 0.455 0.500 -0.333 -0.644
## PpP:PR H_TMHW2: H_TMHW4: PP:H_TMHW2:
## PpP:H_TMHW2
## PpP:H_TMHW4
## PpltnPrt:PR
## Ht_TMHW2:PR 0.512
## Ht_TMHW4:PR 0.500 0.512
## PP:H_TMHW2: -0.731 -0.714 -0.366
## PP:H_TMHW4: -0.707 -0.362 -0.707 0.517
```

```
anova(lmer_SRec_2, type = 3, ddf="Kenward-Roger") #SRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
## Sum Sq Mean Sq NumDF DenDF F value
## Population 0.031158 0.0311576 1 54.769 40.5845
## Heat_Treatment 0.038634 0.0193170 2 2.903 25.1342
## Phase 0.000091 0.0000915 1 57.000 0.1191
## Population:Heat_Treatment 0.002334 0.0011670 2 54.756 1.5201
## Population:Phase 0.005021 0.0050211 1 57.000 6.5402
## Heat_Treatment:Phase 0.012684 0.0063421 2 57.000 8.2609
## Population:Heat_Treatment:Phase 0.001630 0.0008148 2 57.000 1.0613
## Pr(>F)
## Population 4.094e-08 ***
## Heat_Treatment 0.0146885 *
## Phase 0.7312341
## Population:Heat_Treatment 0.2277700
## Population:Phase 0.0132316 *
## Heat_Treatment:Phase 0.0007072 ***
## Population:Heat_Treatment:Phase 0.3527522
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.3. Post-hoc analysis

For the Large MHW:

1. Simple two-way interaction analysis: There was a statistically significant simple two-way interaction between MHW intensity and population for the Recovery period ($F[2, 55] = 202.00$, $p < 0.0001$), but not for the MHW end ($F[2, 55] = 0.03$, $p = 0.969$).
2. Simple-simple main effect analysis: There was a statistically significant simple simple main effect of the MHW intensity on the Fv/Fm for the Atlantic population after the Recovery ($F[2, 3] = 363.00$, $p < 0.001$).

- Simple comparisons: For the Atlantic population, PAM values were only negatively affected by the high intensity MHW after the recovery period (with a strong negative effect, $p < 0.00001$), with values significantly lower than those registered in control individuals.

```

twoway <- function(Data, subset) {
  subset = as.character(subset)
  effects <- lmer(PAM ~ Heat_Treatment * Population + (1 | Tank),
                 data = subset(Data, Phase == subset)) %>%
    anova(., type = 3, ddf="Kenward-Roger") %>%
    broom::tidy() %>%
    mutate(Phase = subset) %>%
    dplyr::select(Phase, dplyr::everything()) %>%
    mutate(Signif = ifelse(p.value < 0.05, "*", ""))

  return(effects)
}

two.way_LRec1 <- twoway(Data = PAM_Stats_LMHW_NO, subset = "MHW")
two.way_LRec2 <- twoway(Data = PAM_Stats_LMHW_NO, subset = "Recovery")
bind_rows(two.way_LRec1, two.way_LRec2)

## # A tibble: 6 x 9
##   Phase    term      sumsq meansq NumDF DenDF statistic  p.value Signif
##   <chr>   <chr>      <dbl>  <dbl> <int> <dbl>      <dbl>    <dbl> <chr>
## 1 MHW     Heat_Treatment  4.09e-3  2.05e-3     2   2.97     3.67  1.58e- 1 ""
## 2 MHW     Population      2.14e-2  2.14e-2     1  55.4    38.4  7.47e- 8 "*"
## 3 MHW     Heat_Treatment~ 3.45e-5  1.73e-5     2  55.4     0.0310 9.69e- 1 ""
## 4 Recovery Heat_Treatment  4.50e-2  2.25e-2     2   2.97    124.  1.37e- 3 "*"
## 5 Recovery Population      1.00e-2  1.00e-2     1  55.3    55.3  7.11e-10 "*"
## 6 Recovery Heat_Treatment~ 7.31e-2  3.66e-2     2  55.3    202.  3.86e-26 "*"

simpleeffects <- function(Data, subset){
  subset = as.character(subset)
  effects <- lmer(PAM ~ Heat_Treatment + (1 | Tank),
                 data = subset(Data, Phase == subset[1] & Population == subset[2])) %>%
    anova(., type = 3, ddf="Kenward-Roger") %>%
    broom::tidy() %>%
    mutate(Phase = subset[1], Population=subset[2]) %>%
    dplyr::select(Phase, Population, dplyr::everything()) %>%
    mutate(Signif = ifelse(p.value < 0.05, "*", ""))

  return(effects)
}

simple_LRec1 <- simpleeffects(Data = PAM_Stats_LMHW_NO, subset = c("MHW", "Cantabria"))
simple_LRec2 <- simpleeffects(Data = PAM_Stats_LMHW_NO, subset = c("Recovery", "Cantabria"))
simple_LRec3 <- simpleeffects(Data = PAM_Stats_LMHW_NO, subset = c("MHW", "Portugal"))
simple_LRec4 <- simpleeffects(Data = PAM_Stats_LMHW_NO, subset = c("Recovery", "Portugal"))
simple_LRec <- bind_rows(simple_LRec1, simple_LRec2, simple_LRec3, simple_LRec4)
simple_LRec

## # A tibble: 4 x 10

```

##	Phase	Population	term	sumsq	meansq	NumDF	DenDF	statistic	p.value	Signif
##	<chr>	<chr>	<chr>	<dbl>	<dbl>	<int>	<dbl>	<dbl>	<dbl>	<chr>
## 1	MHW	Cantabria	Heat~	1.78e-3	8.90e-4	2	2.86	1.68	3.29e-1	" "
## 2	Recovery	Cantabria	Heat~	2.38e-4	1.19e-4	2	2.99	0.785	5.32e-1	" "
## 3	MHW	Portugal	Heat~	2.30e-3	1.15e-3	2	3.03	1.96	2.85e-1	" "
## 4	Recovery	Portugal	Heat~	1.15e-1	5.75e-2	2	3.03	363.	2.46e-4	"*"

```
pwc_LRec <- emmeans(lmer_LRec_2, ~ Heat_Treatment | Phase * Population) %>%
  pairs(adjust="Tukey")
pwc_LRec
```

```
## Phase = MHW, Population = Cantabria:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2 -0.00672 0.00826 28.5  -0.813  0.6979
## Control - MHW4  0.01163 0.00803 26.9   1.447  0.3317
## MHW2 - MHW4     0.01835 0.00844 30.2   2.174  0.0920
##
## Phase = Recovery, Population = Cantabria:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2  0.00401 0.00826 28.5   0.485  0.8791
## Control - MHW4 -0.00971 0.00803 26.9  -1.209  0.4584
## MHW2 - MHW4    -0.01372 0.00844 30.2  -1.625  0.2507
##
## Phase = MHW, Population = Portugal:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2 -0.00731 0.00823 29.4  -0.888  0.6520
## Control - MHW4  0.01450 0.00849 32.1   1.708  0.2177
## MHW2 - MHW4     0.02181 0.00884 36.5   2.466  0.0474
##
## Phase = Recovery, Population = Portugal:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2 -0.00333 0.00823 29.4  -0.405  0.9139
## Control - MHW4  0.13331 0.00849 32.1  15.706 <.0001
## MHW2 - MHW4     0.13665 0.00884 36.5  15.452 <.0001
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

For the Short MHW:

1. Simple two-way interaction analysis: It was not computed considering that there was not significant three-way interactions among treatments. However, the additive combination of two double interactions was significant.
2. Simple-simple main effect analysis: There was a statistically significant simple simple main effect of the MHW intensity on the Fv/Fm values for the Cantabrian population after the MHW (Cantabrian: $F[2, 3] = 11.2$, $p = 0.04$; Atlantic: $F[2, 3] = 8.04$, $p = 0.06$) but not for the Recovery period.
3. Simple comparisons: PAM values were negatively affected by both MHW intensities just after the MHW for the Cantabrian population (with p always below 0.01 comparing to control). Those negative effects only persist after the recovery period ($p < 0.01$ when compared to control) for the high intensity MHW in the Cantabrian population.

```

simple_SRec1 <- simpleeffects(Data = PAM_Stats_SMHW_NO, subset = c("MHW", "Cantabria"))
simple_SRec2 <- simpleeffects(Data = PAM_Stats_SMHW_NO, subset = c("Recovery", "Cantabria"))
simple_SRec3 <- simpleeffects(Data = PAM_Stats_SMHW_NO, subset = c("MHW", "Portugal"))
simple_SRec4 <- simpleeffects(Data = PAM_Stats_SMHW_NO, subset = c("Recovery", "Portugal"))
simple_SRec <- bind_rows(simple_SRec1, simple_SRec2, simple_SRec3, simple_SRec4)
simple_SRec

```

```

## # A tibble: 4 x 10
##   Phase      Population term      sumsq meansq NumDF DenDF statistic p.value Signif
##   <chr>      <chr>      <chr>    <dbl>  <dbl> <int> <dbl>      <dbl>  <dbl> <chr>
## 1 MHW      Cantabria  Heat~  0.0189  0.00944     2  2.90      11.2   0.0434 "*"
## 2 Recovery Cantabria  Heat~  0.00793 0.00396     2  2.86       4.51  0.130 ""
## 3 MHW      Portugal   Heat~  0.0160  0.00800     2  3.02       8.04  0.0617 ""
## 4 Recovery Portugal   Heat~  0.00376 0.00188     2  3.04       2.30  0.247 ""

```

```

pwc_SRec <- emmeans(lmer_SRec_2, ~ Heat_Treatment | Phase * Population) %>%
  pairs(adjust="Tukey")
pwc_SRec

```

```

## Phase = MHW, Population = Cantabria:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2  0.08080 0.0134 28.6   6.050 <.0001
## Control - MHW4  0.05080 0.0137 30.1   3.707  0.0024
## MHW2 - MHW4    -0.03000 0.0134 28.6  -2.246  0.0804
##
## Phase = Recovery, Population = Cantabria:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2  0.03116 0.0134 28.6   2.333  0.0672
## Control - MHW4  0.04843 0.0137 30.1   3.534  0.0037
## MHW2 - MHW4     0.01727 0.0134 28.6   1.293  0.4104
##
## Phase = MHW, Population = Portugal:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2  0.05477 0.0130 27.8   4.205  0.0007
## Control - MHW4  0.05790 0.0136 32.0   4.256  0.0005
## MHW2 - MHW4     0.00313 0.0130 27.8   0.240  0.9687
##
## Phase = Recovery, Population = Portugal:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2  0.00903 0.0130 27.8   0.693  0.7694
## Control - MHW4  0.02690 0.0136 32.0   1.977  0.1342
## MHW2 - MHW4     0.01787 0.0130 27.8   1.372  0.3688
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates

```

P-I curves analysis

1. DATA PROCESSING

Data loading and inspection. First of all, we will visualize raw data using a boxplot.

```
# Data processing
PI <- data.frame(
  read.xlsx("D:/Estancia_Porto_2023/experiments/MHW/Análisis_estadístico/ANOVA_RM/PI/PI_individual_1.xlsx",
    sheet = 2))
PI <- na.omit(PI)
PI <- PI %>% filter(!(Parameter == "Ic" & Value > 150))
PI <- PI[!PI$Parameter == "eopt_estimate",]

## Plot ##
# Labels #

day.labs <- c("After Heatwave", "Recovery")
names(day.labs) <- c("MHW", "Recovery")

Duration.labs <- c("Short Heatwave", "Long Heatwave")
names(Duration.labs) <- c("Srec", "Lrec")

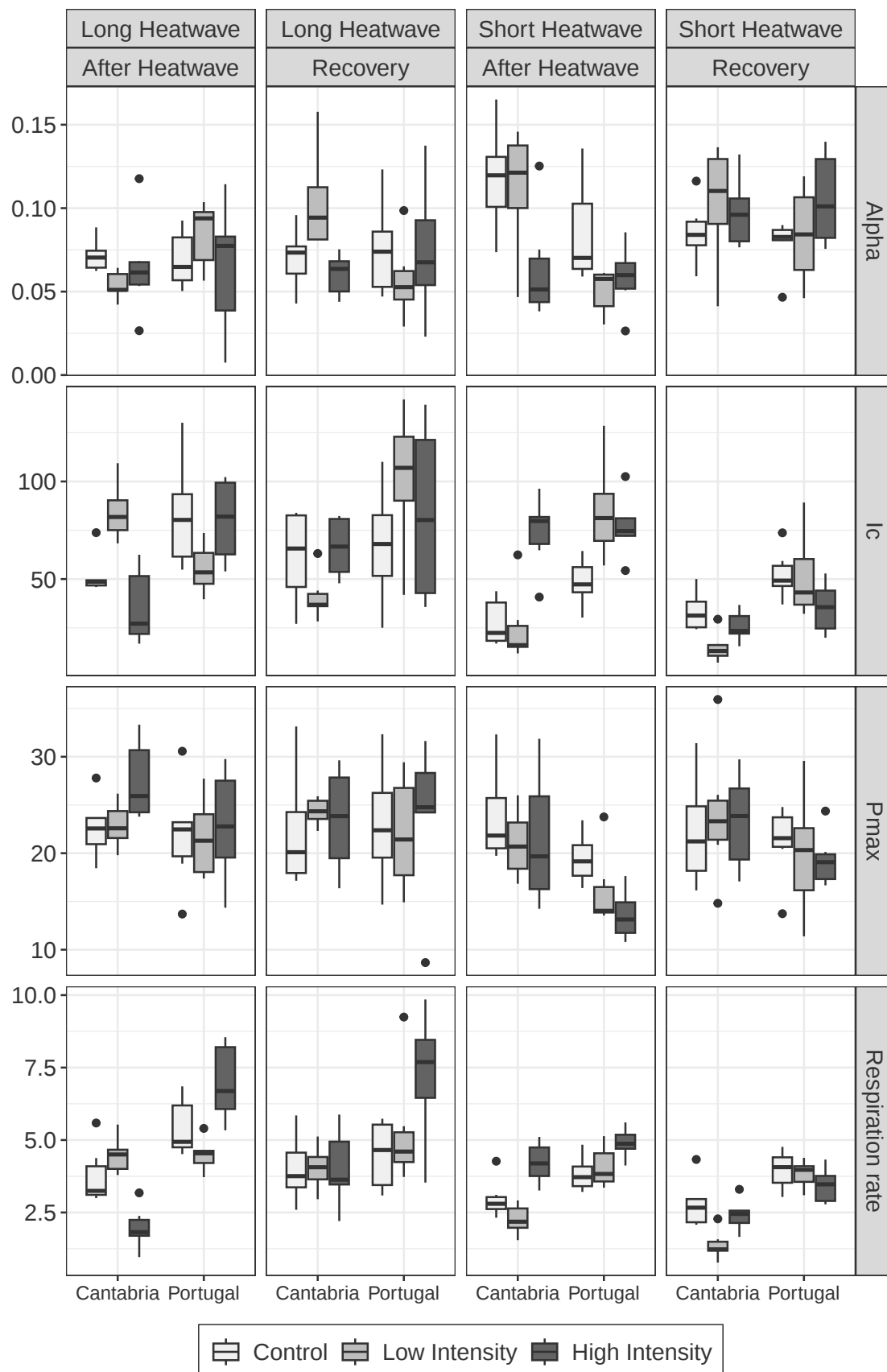
MHW.labs <- c("Control", "Low Intensity", "High Intensity")

Parameters.labs <- c("Alpha", "Eopt", "Pmax", "Respiration rate")
names(Parameters.labs) <- c("alpha_estimate", "eopt_estimate", "ps_estimate", "Resp")

# Plot #
PI_plot <- ggplot(PI, aes(x = as.factor(Population), y = Value,
  fill = as.factor(Heat_Treatment))) +
  geom_boxplot() +
  facet_grid(Parameter~Duration_Treatment*Phase,
    labeller = labeller(Duration_Treatment = Duration.labs,
      Phase = day.labs, Parameter = Parameters.labs),
    scales = "free_y") +
  labs(x = NULL, y = NULL, fill = NULL) +
  theme_bw() +
  theme(axis.text.x = element_text(color = "grey20", size = 10),
    axis.text.y = element_text(color = "grey20", size = 12),
    axis.title.x = element_text(color = "grey20", size = 12),
    axis.title.y = element_text(color = "grey20", size = 12),
    strip.text.x = element_text(color = "grey20", size = 12),
    strip.text.y = element_text(color = "grey20", size = 12),
```

```
legend.text = element_text(color = "grey20", size = 12),  
legend.background = element_rect(colour = "grey20", fill = "white", size = 0.3),  
legend.position="bottom") +  
scale_fill_brewer(palette="Greys", labels = MHW.labs)
```

PI_plot



2. STATISTICAL ANALYSIS

To test the effects of the different treatments (Long/Short, Low/High Intensity MHW) on the photosynthetic parameters estimated from P-I curves, we will use mixed models. The analysis was performed separately for each MHW (Long/Short) as the results were compared to independent control treatments. In this context, **POPULATION** (w/ two levels: Cantabrian and Atlantic) and **MHW INTENSITY** (w/ three levels: Control, Low Intensity and High intensity) were considered between-subject factors, while **TIME** (w/ two levels: after MHW and after recovery period) was considered a within-subject factor. In the mixed model structure, Population, MHW intensity and Time were the fixed factors, while individual ID and Tank were random factors. ID was selected to account for repeated measurements, while Tank was chosen to account for pseudoreplication. The response variables measured were the **Respiration rate**, **alpha** and **Pmax**. The aim was to understand how they change over time (After Stress Period vs Recovery) depending on the population of origin and the induced thermal stress imposed by the MHW (Low vs High vs Non-Stress).

2.1. Alpha analysis

2.1.1. Prepare data

```
Alpha_Stats <- PI[PI$Parameter == "alpha_estimate",]
Alpha_Stats$ID <- as.factor(Alpha_Stats$ID)
Alpha_Stats$Phase <- as.factor(Alpha_Stats$Phase)
Alpha_Stats$Tank <- as.factor(Alpha_Stats$Tank)
Alpha_Stats$Heat_Treatment <- as.factor(Alpha_Stats$Heat_Treatment)
Alpha_Stats$Population <- as.factor(Alpha_Stats$Population)
Alpha_Stats$Value_Log <- log10(Alpha_Stats$Value + 1)
summary(Alpha_Stats)
```

```
##      Day                Phase  Duration_Treatment  Population
## Length:144             MHW      :72  Length:144      Cantabria:72
## Class :character      Recovery:72  Class :character  Portugal :72
## Mode  :character                      Mode  :character
##
##
##
## Heat_Treatment  Sensor                ID      Parameter
## Control:48      Length:144            1      : 4  Length:144
## MHW2   :48      Class :character      2      : 4  Class :character
## MHW4   :48      Mode  :character      3      : 4  Mode  :character
##
##                                     4      : 4
##                                     5      : 4
##                                     6      : 4
##                                     (Other):120
##
##      Value                Tank      Value_Log
## Min.   :0.007456    1      :12  Min.   :0.003226
## 1st Qu.:0.056796    2      :12  1st Qu.:0.023991
## Median :0.074760    3      :12  Median :0.031311
## Mean   :0.078779    4      :12  Mean   :0.032759
## 3rd Qu.:0.097717    5      :12  3rd Qu.:0.040490
## Max.   :0.165089    6      :12  Max.   :0.066359
##                                     (Other):72
```

```
Alpha_Stats_SMHW <- Alpha_Stats[Alpha_Stats$Duration_Treatment == "Srec",]
Alpha_Stats_LMHW <- Alpha_Stats[Alpha_Stats$Duration_Treatment == "Lrec",]
```

2.1.2. Check model assumptions

2.1.2.1. Outliers

First of all, the outliers were identified and removed when considered extreme.

```
# Large MHW
outliers_LMHW_Alpha <- Alpha_Stats_LMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Value)
outliers_LMHW_Alpha <- outliers_LMHW_Alpha[-c(11,12)]
Alpha_Stats_LMHW_NO <- anti_join(Alpha_Stats_LMHW, outliers_LMHW_Alpha, by = c("ID"))
outliers_LMHW_Alpha %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, Value) %>%
  head() # Large MHW outliers
```

```
## # A tibble: 3 x 7
##   Phase      Population Heat_Treatment Day   Duration_Treatment ID      Value
##   <fct>      <fct>      <fct>      <chr> <chr>              <fct> <dbl>
## 1 MHW        Cantabria  MHW4        21    Lrec                7      0.118
## 2 MHW        Cantabria  MHW4        21    Lrec                12     0.0266
## 3 Recovery Portugal  MHW2        28    Lrec                32     0.0986
```

```
#Short MHW
outliers_SMHW_Alpha <- Alpha_Stats_SMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Value)
outliers_SMHW_Alpha <- outliers_SMHW_Alpha[-c(11,12)]
Alpha_Stats_SMHW_NO <- anti_join(Alpha_Stats_SMHW, outliers_SMHW_Alpha, by = c("ID"))
outliers_SMHW_Alpha %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, Value) %>%
  head() # Short MHW outliers
```

```
## # A tibble: 4 x 7
##   Phase      Population Heat_Treatment Day   Duration_Treatment ID      Value
##   <fct>      <fct>      <fct>      <chr> <chr>              <fct> <dbl>
## 1 Recovery Cantabria  Control    14    Srec                5      0.116
## 2 MHW        Cantabria  MHW4        7     Srec                8      0.125
## 3 Recovery Portugal  Control    14    Srec               24     0.0466
## 4 MHW        Portugal  MHW4        7     Srec               30     0.0264
```

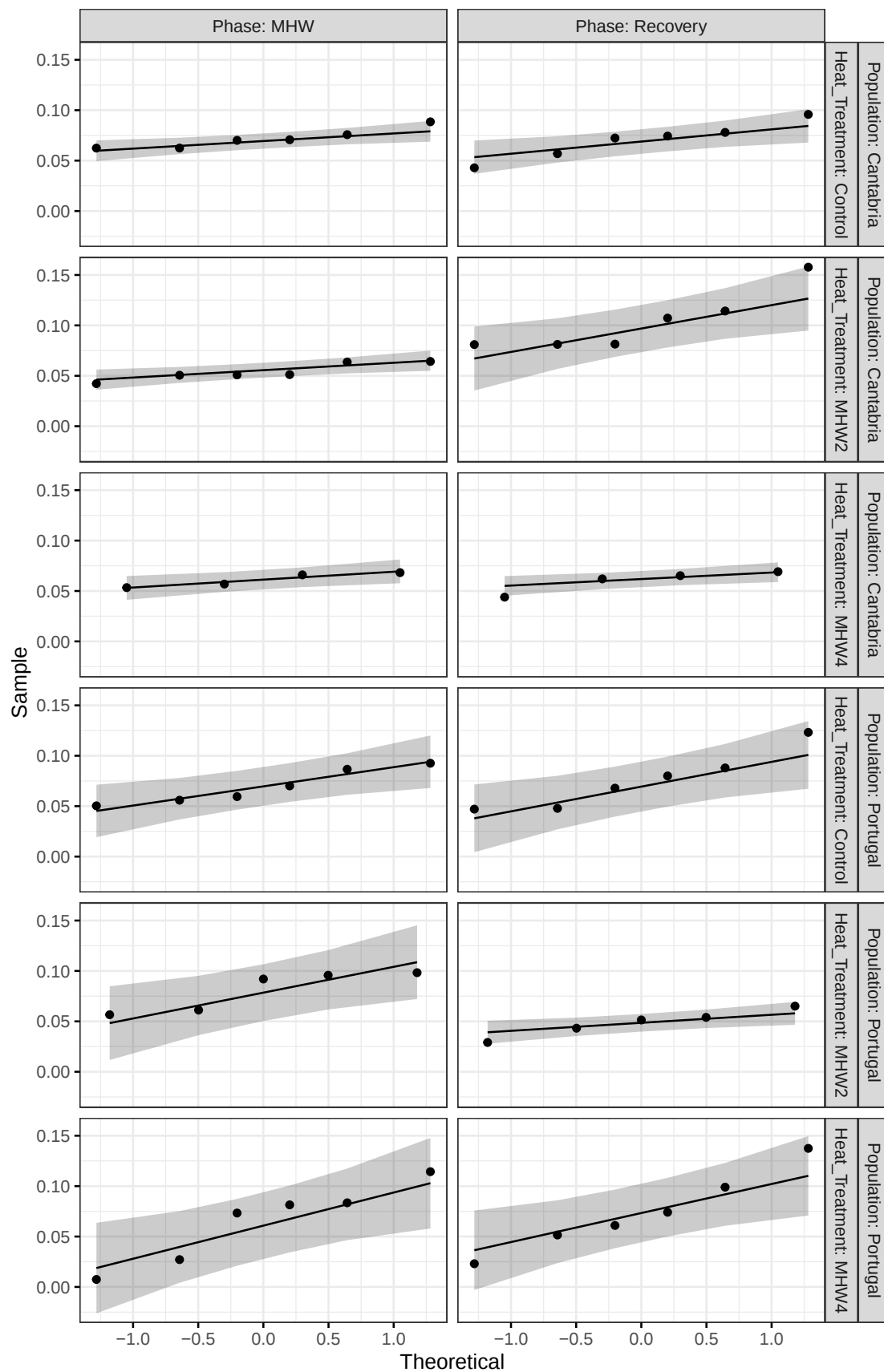
2.1.2.2. Normality

The second step was to test normality. For that purpose, we computed Shapiro-Wilk's tests for each combinations of factor levels. Normality was also assessed visually by inspecting qqplots. According to the results, no clear deviations from normality were observed, with the only exception of the Shapiro test results for the Short Low intensity MHW and Atlantic population. However, it was not that clearly observed in the plot analysis.

```
# Large MMHW
Alpha_Stats_LMHW_NO %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  shapiro_test(Value)
```

```
## # A tibble: 12 x 6
##   Phase      Population Heat_Treatment variable statistic      p
##   <fct>      <fct>      <fct>      <chr>      <dbl> <dbl>
## 1 MHW        Cantabria   Control     Value        0.892 0.330
## 2 Recovery   Cantabria   Control     Value        0.969 0.884
## 3 MHW        Cantabria   MHW2        Value        0.872 0.233
## 4 Recovery   Cantabria   MHW2        Value        0.813 0.0770
## 5 MHW        Cantabria   MHW4        Value        0.902 0.443
## 6 Recovery   Cantabria   MHW4        Value        0.855 0.244
## 7 MHW        Portugal    Control     Value        0.913 0.453
## 8 Recovery   Portugal    Control     Value        0.920 0.505
## 9 MHW        Portugal    MHW2        Value        0.800 0.0809
## 10 Recovery   Portugal    MHW2        Value        0.980 0.932
## 11 MHW        Portugal    MHW4        Value        0.925 0.541
## 12 Recovery   Portugal    MHW4        Value        0.979 0.945
```

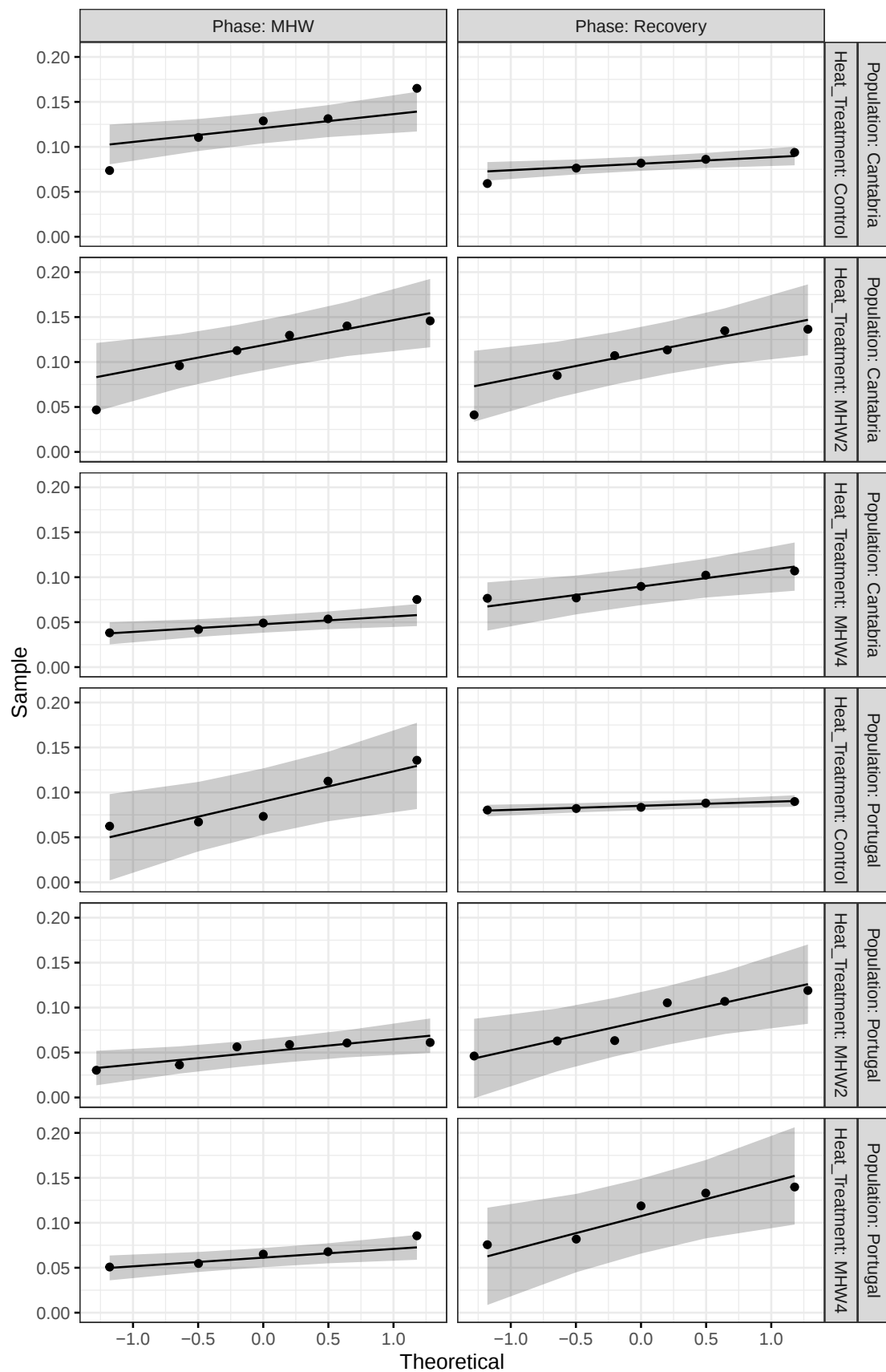
```
ggqqplot(Alpha_Stats_LMHW_NO, "Value", ggtheme = theme_bw()) +
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")
```



```
# Short MMHW
Alpha_Stats_SMHW_NO %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  shapiro_test(Value)
```

```
## # A tibble: 12 x 6
##   Phase      Population Heat_Treatment variable statistic      p
##   <fct>      <fct>      <fct>      <chr>      <dbl> <dbl>
## 1 MHW        Cantabria   Control     Value        0.971 0.880
## 2 Recovery   Cantabria   Control     Value        0.951 0.741
## 3 MHW        Cantabria   MHW2        Value        0.890 0.319
## 4 Recovery   Cantabria   MHW2        Value        0.897 0.359
## 5 MHW        Cantabria   MHW4        Value        0.893 0.374
## 6 Recovery   Cantabria   MHW4        Value        0.879 0.305
## 7 MHW        Portugal    Control     Value        0.856 0.214
## 8 Recovery   Portugal    Control     Value        0.919 0.521
## 9 MHW        Portugal    MHW2        Value        0.778 0.0371
## 10 Recovery  Portugal    MHW2        Value        0.878 0.259
## 11 MHW        Portugal    MHW4        Value        0.935 0.628
## 12 Recovery  Portugal    MHW4        Value        0.872 0.276
```

```
ggqqplot(Alpha_Stats_SMHW_NO, "Value", ggtheme = theme_bw()) +
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")
```



2.1.2.3. Homogeneity of variances

Homogeneity of variances was checked through Levene's tests. Tests using the complete dataset did not reveal heteroscedasticity ($p > 0.05$) for any MHW duration (Large/Short).

```
#Large MHW
```

```
Alpha_Stats_LMHW_NO %>%  
  levene_test(Value ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4  
##   df1 df2 statistic    p  
##   <int> <int>     <dbl> <dbl>  
## 1    11    54      1.64 0.114
```

```
Alpha_Stats_LMHW_NO %>%  
  group_by(Phase) %>%  
  levene_test(Value ~ Heat_Treatment*Population)
```

```
## # A tibble: 2 x 5  
##   Phase    df1 df2 statistic    p  
##   <fct>   <int> <int>     <dbl> <dbl>  
## 1 MHW         5    27      2.03 0.106  
## 2 Recovery    5    27      1.36 0.271
```

```
Alpha_Stats_LMHW_NO %>%  
  group_by(Phase, Population) %>%  
  levene_test(Value ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6  
##   Phase    Population    df1 df2 statistic    p  
##   <fct>   <fct>      <int> <int>     <dbl> <dbl>  
## 1 MHW    Cantabria      2    13     0.0313 0.969  
## 2 MHW    Portugal       2    14     0.982 0.399  
## 3 Recovery Cantabria      2    13     1.35 0.293  
## 4 Recovery Portugal       2    14     1.58 0.241
```

```
#Short MHW
```

```
Alpha_Stats_SMHW_NO %>%  
  levene_test(Value ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4  
##   df1 df2 statistic    p  
##   <int> <int>     <dbl> <dbl>  
## 1    11    52      1.46 0.175
```

```
Alpha_Stats_SMHW_NO %>%  
  group_by(Phase) %>%  
  levene_test(Value ~ Heat_Treatment*Population)
```

```
## # A tibble: 2 x 5  
##   Phase    df1 df2 statistic    p  
##   <fct>   <int> <int>     <dbl> <dbl>  
## 1 MHW         5    26      1.01 0.432  
## 2 Recovery    5    26      2.74 0.0407
```

```
Alpha_Stats_SMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(Value ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Phase      Population    df1    df2 statistic      p
##   <fct>      <fct>      <int> <int>     <dbl>  <dbl>
## 1 MHW        Cantabria         2     13     0.964 0.407
## 2 MHW        Portugal          2     13     1.20 0.333
## 3 Recovery  Cantabria         2     13     1.69 0.223
## 4 Recovery  Portugal          2     13     7.02 0.00855
```

2.1.3. Mixed model

From the model results for the Short MHW what we observed was that there was not a statistically significant ($p > .05$) three-way interaction between time, population, and MHW intensity ($F[2, 26] = 0.794$, $p = 0.463$), but there were significant two-way interactions between Population/MHW intensity ($F[2, 26] = 6.40$, $p = 0.006$), Population/time ($F[1, 26] = 5.107$, $p = 0.032$) and MHW intensity/time ($F[2, 26] = 8.694$, $p = 0.001$). For the Large MHW, a significant three-way interaction was detected ($F[2, 27] = 18.60$, $p < 0.001$).

```
#LRec: Repeated measures + random tank effects
lmer_LRec_Alpha <- lmerTest::lmer(Value ~ Population*Heat_Treatment*Phase
                                + (1 | Tank) + (1 | ID),
                                Alpha_Stats_LMHW_NO, REML = TRUE)
summary(lmer_LRec_Alpha, ddf="Kenward-Roger") #LRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: Value ~ Population * Heat_Treatment * Phase + (1 | Tank) + (1 |
## ID)
## Data: Alpha_Stats_LMHW_NO
##
## REML criterion at convergence: -246.3
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.31046 -0.50822 -0.02393  0.37312  2.05779
##
## Random effects:
##   Groups   Name                Variance Std.Dev.
##   ID       (Intercept)  0.0002721  0.01650
##   Tank     (Intercept)  0.0001784  0.01336
##   Residual                    0.0002042  0.01429
## Number of obs: 66, groups: ID, 33; Tank, 6
##
## Fixed effects:
##                                     Estimate Std. Error
## (Intercept)                       0.071641    0.012984
## PopulationPortugal                 -0.002468    0.012601
## Heat_TreatmentMHW2                 -0.017853    0.018362
## Heat_TreatmentMHW4                 -0.010541    0.019413
## PhaseRecovery                     -0.001596    0.008251
```

```

## PopulationPortugal:Heat_TreatmentMHW2          0.030696    0.018304
## PopulationPortugal:Heat_TreatmentMHW4          0.005890    0.018901
## PopulationPortugal:PhaseRecovery               0.008066    0.011668
## Heat_TreatmentMHW2:PhaseRecovery               0.051606    0.011668
## Heat_TreatmentMHW4:PhaseRecovery               0.000556    0.013045
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery -0.090296    0.016909
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 0.002794    0.017502
##
## df t value Pr(>|t|)
## (Intercept)          5.539753    5.518 0.001932
## PopulationPortugal   36.471601   -0.196 0.845804
## Heat_TreatmentMHW2    5.539753   -0.972 0.371420
## Heat_TreatmentMHW4    6.850737   -0.543 0.604347
## PhaseRecovery        27.000000   -0.193 0.848080
## PopulationPortugal:Heat_TreatmentMHW2          36.529575    1.677 0.102076
## PopulationPortugal:Heat_TreatmentMHW4          36.471601    0.312 0.757087
## PopulationPortugal:PhaseRecovery              27.000000    0.691 0.495287
## Heat_TreatmentMHW2:PhaseRecovery              27.000000    4.423 0.000143
## Heat_TreatmentMHW4:PhaseRecovery              27.000000    0.043 0.966315
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 27.000000   -5.340 1.22e-05
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 27.000000    0.160 0.874363
##
## (Intercept)          **
## PopulationPortugal
## Heat_TreatmentMHW2
## Heat_TreatmentMHW4
## PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW2
## PopulationPortugal:Heat_TreatmentMHW4
## PopulationPortugal:PhaseRecovery
## Heat_TreatmentMHW2:PhaseRecovery          ***
## Heat_TreatmentMHW4:PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery ***
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PopltnPrtgl -0.485
## Ht_TrtnMHW2 -0.707  0.343
## Ht_TrtnMHW4 -0.669  0.325  0.473
## PhaseRecvry -0.318  0.327  0.225   0.213
## PpP:H_TMHW2  0.334 -0.689 -0.473  -0.224  -0.226
## PpP:H_TMHW4  0.324 -0.667 -0.229  -0.541  -0.218  0.459
## PpltnPrt:PR  0.225 -0.463 -0.159  -0.150  -0.707  0.319   0.309
## Ht_TMHW2:PR  0.225 -0.231 -0.318  -0.150  -0.707  0.319   0.154
## Ht_TMHW4:PR  0.201 -0.207 -0.142  -0.336  -0.632  0.143   0.345
## PP:H_TMHW2: -0.155  0.319  0.219   0.104   0.488 -0.462  -0.213
## PP:H_TMHW4: -0.150  0.309  0.106   0.250   0.471 -0.213  -0.463
##
## PpP:PR H_TMHW2: H_TMHW4: PP:H_TMHW2:
## PopltnPrtgl
## Ht_TrtnMHW2
## Ht_TrtnMHW4
## PhaseRecvry

```

```
## PpP:H_TMHW2
## PpP:H_TMHW4
## PpltnPrt:PR
## Ht_TMHW2:PR 0.500
## Ht_TMHW4:PR 0.447 0.447
## PP:H_TMHW2: -0.690 -0.690 -0.309
## PP:H_TMHW4: -0.667 -0.333 -0.745 0.460
```

```
anova(lmer_LRec_Alpha, type = 3, ddf="Kenward-Roger") #LRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##
##              Sum Sq   Mean Sq NumDF   DenDF F value
## Population      0.0000030 0.0000030     1 24.0455  0.0145
## Heat_Treatment  0.0000530 0.0000265     2  3.0232  0.1296
## Phase           0.0004427 0.0004427     1 27.0000  2.1680
## Population:Heat_Treatment 0.0003458 0.0001729     2 24.0458  0.8466
## Population:Phase 0.0017944 0.0017944     1 27.0000  8.7866
## Heat_Treatment:Phase 0.0001234 0.0000617     2 27.0000  0.3022
## Population:Heat_Treatment:Phase 0.0075970 0.0037985     2 27.0000 18.6006
##
##              Pr(>F)
## Population      0.904997
## Heat_Treatment  0.883053
## Phase           0.152470
## Population:Heat_Treatment 0.441261
## Population:Phase 0.006271 **
## Heat_Treatment:Phase 0.741671
## Population:Heat_Treatment:Phase 8.348e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#SRec: Repeated measures + random tank effects
```

```
lmer_SRec_Alpha <- lmerTest::lmer(Value ~ Population*Heat_Treatment*Phase
+ (1 | Tank) + (1 | ID),
                                Alpha_Stats_SMHW_NO, REML = TRUE)
summary(lmer_SRec_Alpha, ddf="Kenward-Roger") #SRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: Value ~ Population * Heat_Treatment * Phase + (1 | Tank) + (1 |
## ID)
## Data: Alpha_Stats_SMHW_NO
##
## REML criterion at convergence: -214.1
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.54591 -0.56021  0.08673  0.54369  1.70958
##
## Random effects:
## Groups   Name                Variance Std.Dev.
## ID       (Intercept) 1.289e-05 0.003591
## Tank     (Intercept) 1.807e-05 0.004251
## Residual                            6.267e-04 0.025034
```

```

## Number of obs: 64, groups: ID, 32; Tank, 6
##
## Fixed effects:
##
##                                     Estimate Std. Error
## (Intercept)                        0.121434    0.011775
## PopulationPortugal                 -0.031677    0.015995
## Heat_TreatmentMHW2                 -0.009597    0.015947
## Heat_TreatmentMHW4                 -0.069681    0.016653
## PhaseRecovery                     -0.042408    0.015833
## PopulationPortugal:Heat_TreatmentMHW2 -0.029561    0.021657
## PopulationPortugal:Heat_TreatmentMHW4  0.044498    0.022771
## PopulationPortugal:PhaseRecovery      0.036958    0.022391
## Heat_TreatmentMHW2:PhaseRecovery      0.033586    0.021438
## Heat_TreatmentMHW4:PhaseRecovery      0.081347    0.022391
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 0.005180    0.030318
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery -0.030883    0.031666
##
##                                     df t value Pr(>|t|)
## (Intercept)                     24.478563  10.313 2.16e-10
## PopulationPortugal              48.700455  -1.980 0.053319
## Heat_TreatmentMHW2             22.267724  -0.602 0.553392
## Heat_TreatmentMHW4             24.478563  -4.184 0.000319
## PhaseRecovery                   26.000000  -2.678 0.012650
## PopulationPortugal:Heat_TreatmentMHW2 48.700455  -1.365 0.178548
## PopulationPortugal:Heat_TreatmentMHW4 49.464366   1.954 0.056347
## PopulationPortugal:PhaseRecovery 26.000000   1.651 0.110856
## Heat_TreatmentMHW2:PhaseRecovery 26.000000   1.567 0.129284
## Heat_TreatmentMHW4:PhaseRecovery 26.000000   3.633 0.001208
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 26.000000   0.171 0.865665
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 26.000000  -0.975 0.338402
##
## (Intercept)                    ***
## PopulationPortugal              .
## Heat_TreatmentMHW2
## Heat_TreatmentMHW4            ***
## PhaseRecovery                  *
## PopulationPortugal:Heat_TreatmentMHW2
## PopulationPortugal:Heat_TreatmentMHW4 .
## PopulationPortugal:PhaseRecovery
## Heat_TreatmentMHW2:PhaseRecovery
## Heat_TreatmentMHW4:PhaseRecovery **
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PpP:H_TMHW2 -0.683
## Ht_TrtmMHW2 -0.737  0.503
## Ht_TrtmMHW4 -0.707  0.483  0.521
## PhaseRecvry -0.676  0.495  0.498   0.478
## PpP:H_TMHW2  0.504 -0.739 -0.681  -0.357  -0.366
## PpP:H_TMHW4  0.482 -0.706 -0.355  -0.683  -0.350  0.522
## PpltnPrt:PR  0.478 -0.700 -0.352  -0.338  -0.707  0.517   0.494

```

```
## Ht_TMHw2:PR 0.499 -0.366 -0.674 -0.353 -0.739 0.495 0.258
## Ht_TMHw4:PR 0.478 -0.350 -0.352 -0.676 -0.707 0.258 0.494
## PP:H_TMHw2: -0.353 0.517 0.477 0.250 0.522 -0.700 -0.365
## PP:H_TMHw4: -0.338 0.495 0.249 0.478 0.500 -0.366 -0.699
## PpP:PR H_TMHw2: H_TMHw4: PP:H_TMHw2:
## PopltnPrtgl
## Ht_TrtmMHW2
## Ht_TrtmMHW4
## PhaseRecvry
## PpP:H_TMHw2
## PpP:H_TMHw4
## PpltnPrt:PR
## Ht_TMHw2:PR 0.522
## Ht_TMHw4:PR 0.500 0.522
## PP:H_TMHw2: -0.739 -0.707 -0.369
## PP:H_TMHw4: -0.707 -0.369 -0.707 0.522
```

```
anova(lmer_SRec_Alpha, type = 3, ddf="Kenward-Roger") #SRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
## Sum Sq Mean Sq NumDF DenDF F value
## Population 0.0023413 0.0023413 1 23.5055 3.7359
## Heat_Treatment 0.0015584 0.0007792 2 2.8759 1.2418
## Phase 0.0016196 0.0016196 1 26.0000 2.5843
## Population:Heat_Treatment 0.0080104 0.0040052 2 23.4791 6.3906
## Population:Phase 0.0032003 0.0032003 1 26.0000 5.1066
## Heat_Treatment:Phase 0.0108976 0.0054488 2 26.0000 8.6944
## Population:Heat_Treatment:Phase 0.0009948 0.0004974 2 26.0000 0.7937
## Pr(>F)
## Population 0.065390 .
## Heat_Treatment 0.408555
## Phase 0.120007
## Population:Heat_Treatment 0.006083 **
## Population:Phase 0.032442 *
## Heat_Treatment:Phase 0.001285 **
## Population:Heat_Treatment:Phase 0.462835
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.1.4. Post-HOC analysis

For the Large MHW:

1. Simple two-way interaction analysis: There was a statistically significant simple two-way interaction between MHW intensity and population for the Recovery period ($F[2, 24] = 6.28$, $p = 0.006$), but not for the MHW end ($F[2, 24] = 2.06$, $p = 0.149$).
2. Simple-simple main effect analysis: No significant effects.
3. Simple comparisons: No significant differences.

```

twoway <- function(Data, subset) {
  subset = as.character(subset)
  effects <- lmer(Value ~ Heat_Treatment * Population + (1 | Tank),
    data = subset(Data, Phase == subset)) %>%
    anova(., type = 3, ddf="Kenward-Roger") %>%
    broom::tidy() %>%
    mutate(Phase = subset) %>%
    dplyr::select(Phase, dplyr::everything()) %>%
    mutate(Signif = ifelse(p.value < 0.05, "*", ""))

  return(effects)
}

twoway2 <- function(Data, subset) {
  subset = as.character(subset)
  effects <- lmer(Value_Log ~ Heat_Treatment * Population + (1 | Tank),
    data = subset(Data, Phase == subset)) %>%
    anova(., type = 3, ddf="Kenward-Roger") %>%
    broom::tidy() %>%
    mutate(Phase = subset) %>%
    dplyr::select(Phase, dplyr::everything()) %>%
    mutate(Signif = ifelse(p.value < 0.05, "*", ""))

  return(effects)
}

two.way_LRec1_Alpha <- twoway(Data = Alpha_Stats_LMHW_NO, subset = "MHW")
two.way_LRec2_Alpha <- twoway(Data = Alpha_Stats_LMHW_NO, subset = "Recovery")
bind_rows(two.way_LRec1_Alpha, two.way_LRec2_Alpha) #LRec

## # A tibble: 6 x 9
##   Phase   term      sumsq  meansq NumDF DenDF statistic p.value Signif
##   <chr>   <chr>      <dbl>   <dbl> <int> <dbl>      <dbl>   <dbl> <chr>
## 1 MHW     Heat_Treatment  8.64e-5  4.32e-5     2   3.02     0.125  0.887   ""
## 2 MHW     Population     7.37e-4  7.37e-4     1  24.0     2.14   0.156   ""
## 3 MHW     Heat_Treatment:~ 1.42e-3  7.10e-4     2  24.0     2.06   0.149   ""
## 4 Recovery Heat_Treatment  1.60e-4  8.02e-5     2   3.03     0.139  0.876   ""
## 5 Recovery Population     1.02e-3  1.02e-3     1  24.1     1.76   0.197   ""
## 6 Recovery Heat_Treatment:~ 7.26e-3  3.63e-3     2  24.1     6.28   0.00638 "*"

simpleeffects <- function(Data, subset){
  subset = as.character(subset)
  effects <- lmer(Value ~ Heat_Treatment + (1 | Tank),
    data = subset(Data, Phase == subset[1] & Population == subset[2])) %>%
    anova(., type = 3, ddf="Kenward-Roger") %>%
    broom::tidy() %>%
    mutate(Phase = subset[1], Population=subset[2]) %>%
    dplyr::select(Phase, Population, dplyr::everything()) %>%
    mutate(Signif = ifelse(p.value < 0.05, "*", ""))

  return(effects)
}

```

```

simpleeffects2 <- function(Data, subset){
  subset = as.character(subset)
  effects <- lmer(Value_Log ~ Heat_Treatment + (1 | Tank),
                  data = subset(Data, Phase == subset[1] & Population == subset[2])) %>%
    anova(., type = 3, ddf="Kenward-Roger") %>%
    broom::tidy() %>%
    mutate(Phase = subset[1], Population=subset[2]) %>%
    dplyr::select(Phase, Population, dplyr::everything()) %>%
    mutate(Signif = ifelse(p.value < 0.05, "*", ""))

  return(effects)
}

simple_LRec1_Alpha <- simpleeffects(Data = Alpha_Stats_LMHW_NO,
                                   subset = c("MHW", "Cantabria"))
simple_LRec2_Alpha <- simpleeffects(Data = Alpha_Stats_LMHW_NO,
                                   subset = c("Recovery", "Cantabria"))
simple_LRec3_Alpha <- simpleeffects(Data = Alpha_Stats_LMHW_NO,
                                   subset = c("MHW", "Portugal"))
simple_LRec4_Alpha <- simpleeffects(Data = Alpha_Stats_LMHW_NO,
                                   subset = c("Recovery", "Portugal"))
simple_LRec_Alpha <- bind_rows(simple_LRec1_Alpha, simple_LRec2_Alpha,
                              simple_LRec3_Alpha, simple_LRec4_Alpha)
simple_LRec_Alpha

## # A tibble: 4 x 10
##   Phase      Population term      sumsq meansq NumDF DenDF statistic p.value Signif
##   <chr>      <chr>      <chr>    <dbl>  <dbl> <int> <dbl>    <dbl>  <dbl> <chr>
## 1 MHW      Cantabria Heat~  9.64e-4 4.82e-4     2   3.13     6.24  0.0809 ""
## 2 Recovery Cantabria Heat~  3.70e-3 1.85e-3     2   3.07     4.06  0.138 ""
## 3 MHW      Portugal  Heat~  2.19e-4 1.10e-4     2   2.99     0.242 0.799 ""
## 4 Recovery Portugal  Heat~  1.17e-3 5.83e-4     2   2.97     0.783 0.533 ""

pwc_LRec_Alpha <- emmeans(lmer_LRec_Alpha, ~ Heat_Treatment | Phase * Population) %>%
  pairs(adjust="Tukey")
pwc_LRec_Alpha

## Phase = MHW, Population = Cantabria:
## contrast      estimate      SE   df t.ratio p.value
## Control - MHW2  0.01785 0.0184 5.54   0.972  0.6209
## Control - MHW4  0.01054 0.0194 6.85   0.543  0.8533
## MHW2 - MHW4    -0.00731 0.0194 6.85  -0.377  0.9256
##
## Phase = Recovery, Population = Cantabria:
## contrast      estimate      SE   df t.ratio p.value
## Control - MHW2 -0.03375 0.0184 5.54  -1.838  0.2421
## Control - MHW4  0.00998 0.0194 6.85   0.514  0.8670
## MHW2 - MHW4     0.04374 0.0194 6.85   2.253  0.1307
##
## Phase = MHW, Population = Portugal:
## contrast      estimate      SE   df t.ratio p.value

```

```
## Control - MHW2 -0.01284 0.0188 6.07 -0.682 0.7820
## Control - MHW4 0.00465 0.0184 5.54 0.253 0.9655
## MHW2 - MHW4 0.01749 0.0188 6.07 0.929 0.6433
##
## Phase = Recovery, Population = Portugal:
## contrast estimate SE df t.ratio p.value
## Control - MHW2 0.02585 0.0188 6.07 1.373 0.4105
## Control - MHW4 0.00130 0.0184 5.54 0.071 0.9972
## MHW2 - MHW4 -0.02455 0.0188 6.07 -1.304 0.4431
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

For the Short MHW:

1. Simple two-way interaction analysis: It was not computed considering that there was not significant three-way interactions among treatments. However, the additive combination of all double interactions was significant.
2. Simple-simple main effect analysis: No clear significant effects.
3. Simple comparisons: Alpha was only significantly reduced by the high intensity MHW for the Cantabrian population ($p = 0.001$) after the MHW.

```
simple_SRec1_Alpha <- simpleeffects(Data = Alpha_Stats_SMHW_NO,
                                   subset = c("MHW", "Cantabria"))
simple_SRec2_Alpha <- simpleeffects(Data = Alpha_Stats_SMHW_NO,
                                   subset = c("Recovery", "Cantabria"))
simple_SRec3_Alpha <- simpleeffects(Data = Alpha_Stats_SMHW_NO,
                                   subset = c("MHW", "Portugal"))
simple_SRec4_Alpha <- simpleeffects(Data = Alpha_Stats_SMHW_NO,
                                   subset = c("Recovery", "Portugal"))
simple_SRec_Alpha <- bind_rows(simple_SRec1_Alpha, simple_SRec2_Alpha,
                              simple_SRec3_Alpha, simple_SRec4_Alpha)
simple_SRec_Alpha

## # A tibble: 4 x 10
## Phase Population term sumsq meansq NumDF DenDF statistic p.value Signif
## <chr> <chr> <chr> <dbl> <dbl> <int> <dbl> <dbl> <dbl> <chr>
## 1 MHW Cantabria Heat~ 0.0140 6.98e-3 2 2.76 7.51 0.0766 ""
## 2 Recovery Cantabria Heat~ 0.00148 7.39e-4 2 2.76 1.22 0.417 ""
## 3 MHW Portugal Heat~ 0.00170 8.50e-4 2 2.94 2.39 0.242 ""
## 4 Recovery Portugal Heat~ 0.00213 1.06e-3 2 2.76 1.72 0.328 ""

pwc_SRec_Alpha <- emmeans(lmer_SRec_Alpha, ~ Heat_Treatment | Phase * Population) %>%
  pairs(adjust="Tukey")
pwc_SRec_Alpha

## Phase = MHW, Population = Cantabria:
## contrast estimate SE df t.ratio p.value
## Control - MHW2 0.009597 0.0159 22.3 0.602 0.8206
## Control - MHW4 0.069681 0.0167 24.5 4.184 0.0009
## MHW2 - MHW4 0.060084 0.0159 22.3 3.768 0.0029
```

```
##
## Phase = Recovery, Population = Cantabria:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2 -0.023989 0.0159 22.3  -1.504  0.3080
## Control - MHW4 -0.011666 0.0167 24.5  -0.701  0.7654
## MHW2 - MHW4     0.012323 0.0159 22.3   0.773  0.7232
##
## Phase = MHW, Population = Portugal:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2  0.039157 0.0159 22.3   2.456  0.0559
## Control - MHW4  0.025183 0.0167 24.5   1.512  0.3028
## MHW2 - MHW4    -0.013974 0.0159 22.3  -0.876  0.6604
##
## Phase = Recovery, Population = Portugal:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2  0.000391 0.0159 22.3   0.025  0.9997
## Control - MHW4 -0.025280 0.0167 24.5  -1.518  0.3001
## MHW2 - MHW4    -0.025672 0.0159 22.3  -1.610  0.2624
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

2.2. Maximum photosynthetic rate (Pmax)

2.2.1. Prepare data

```
Pmax_Stats <- PI[PI$Parameter == "ps_estimate",]
Pmax_Stats$ID <- as.factor(Pmax_Stats$ID)
Pmax_Stats$Phase <- as.factor(Pmax_Stats$Phase)
Pmax_Stats$Tank <- as.factor(Pmax_Stats$Tank)
Pmax_Stats$Heat_Treatment <- as.factor(Pmax_Stats$Heat_Treatment)
Pmax_Stats$Population <- as.factor(Pmax_Stats$Population)
Pmax_Stats$Value_Log <- log10(Pmax_Stats$Value + 1)
summary(Pmax_Stats)
```

```
##      Day      Phase  Duration_Treatment  Population
## Length:144      MHW      :72  Length:144      Cantabria:72
## Class :character  Recovery:72  Class :character  Portugal :72
## Mode  :character                      Mode  :character
##
##
##
## Heat_Treatment  Sensor      ID      Parameter
## Control:48      Length:144    1      : 4  Length:144
## MHW2   :48      Class :character 2      : 4  Class :character
## MHW4   :48      Mode  :character 3      : 4  Mode  :character
##
##
##
##
##      (Other):120
##      Value      Tank      Value_Log
```

```
## Min.      : 8.661    1      :12    Min.      :0.985
## 1st Qu.:18.066    2      :12    1st Qu.:1.280
## Median :21.885    3      :12    Median :1.360
## Mean    :21.816    4      :12    Mean    :1.346
## 3rd Qu.:25.064    5      :12    3rd Qu.:1.416
## Max.     :35.934    6      :12    Max.     :1.567
##                                     (Other):72
```

```
Pmax_Stats_SMHW <- Pmax_Stats[Pmax_Stats$Duration_Treatment == "Srec",]
Pmax_Stats_LMHW <- Pmax_Stats[Pmax_Stats$Duration_Treatment == "Lrec",]
```

2.2.2. Check Model assumptions

2.2.2.1. Outliers

First of all, the outliers were identified and removed when considered extreme.

```
# Large MHW
outliers_LMHW_Pmax <- Pmax_Stats_LMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Value_Log)
outliers_LMHW_Pmax <- outliers_LMHW_Pmax[-c(11,12)]
Pmax_Stats_LMHW_NO <- anti_join(Pmax_Stats_LMHW, outliers_LMHW_Pmax, by = c("ID"))
outliers_LMHW_Pmax %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, Value) %>%
  head() # Large MHW outliers
```

```
## # A tibble: 3 x 7
##   Phase      Population Heat_Treatment Day   Duration_Treatment ID      Value
##   <fct>      <fct>      <fct>      <chr> <chr>              <fct> <dbl>
## 1 MHW        Portugal    Control    21     Lrec              20     13.7
## 2 MHW        Portugal    Control    21     Lrec              23     30.6
## 3 Recovery Portugal    MHW4       28     Lrec              28      8.66
```

```
# Short MMHW
outliers_SMHW_Pmax <- Pmax_Stats_SMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Value)
outliers_SMHW_Pmax <- outliers_SMHW_Pmax[-c(11,12)]
Pmax_Stats_SMHW_NO <- anti_join(Pmax_Stats_SMHW, outliers_SMHW_Pmax, by = c("ID"))
outliers_SMHW_Pmax %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, Value) %>%
  head() # Short MHW outliers
```

```
## # A tibble: 5 x 7
##   Phase      Population Heat_Treatment Day   Duration_Treatment ID      Value
##   <fct>      <fct>      <fct>      <chr> <chr>              <fct> <dbl>
## 1 Recovery Cantabria  MHW2       14     Srec              13     14.8
## 2 Recovery Cantabria  MHW2       14     Srec              17     35.9
## 3 Recovery Portugal   Control    14     Srec              24     13.7
## 4 MHW        Portugal   MHW2       7      Srec              35     23.8
## 5 Recovery Portugal   MHW4      14     Srec              25     24.4
```

2.2.2.2. Normality

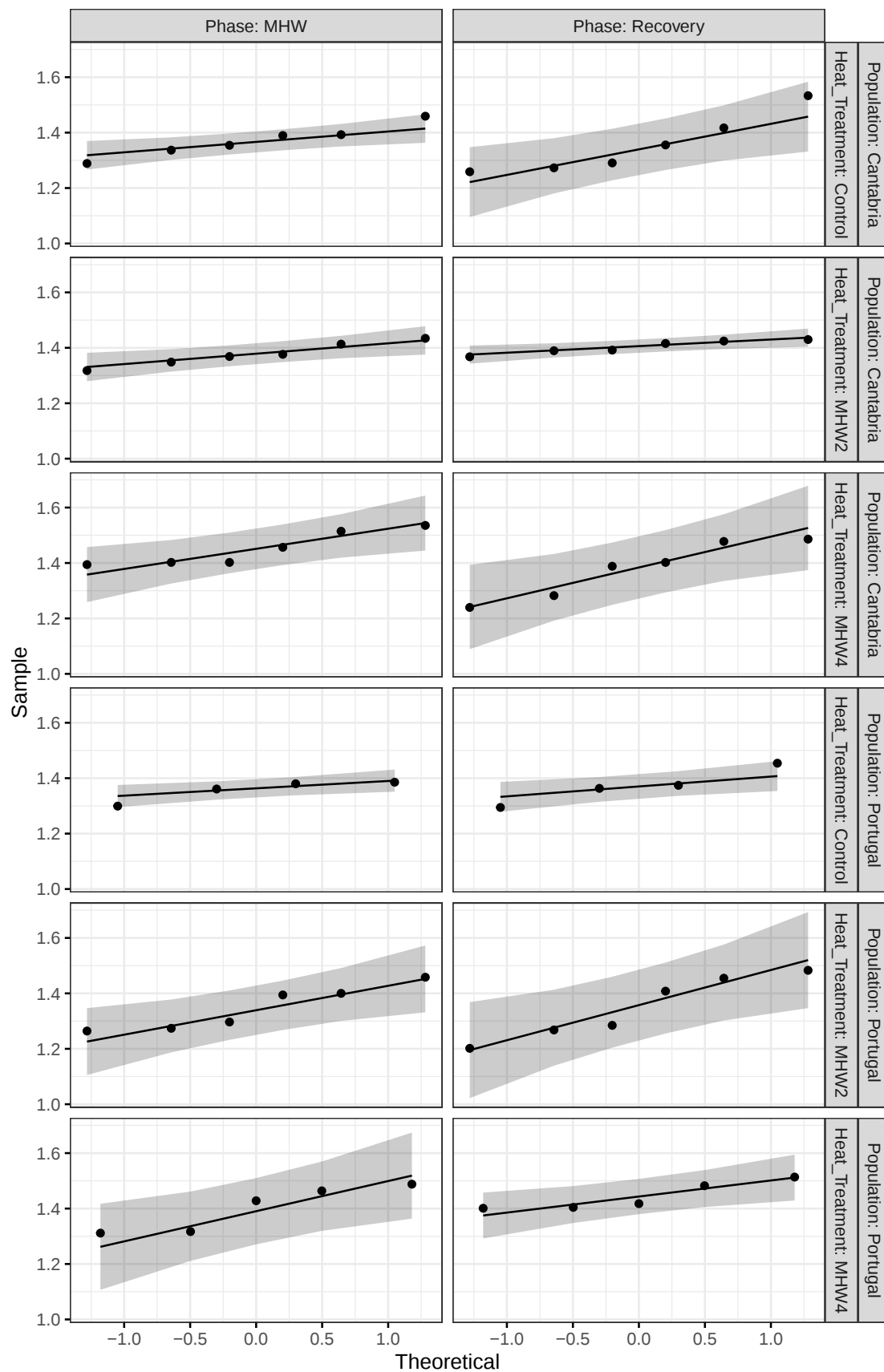
The second step was to test normality. For that purpose, we computed Shapiro-Wilk's tests for each combinations of factor levels. Normality was also assessed visually by inspecting qqplots. According to the results, no clear deviations from normality were observed, with the only exception of the Shapiro test results for the Short Low intensity MHW and Atlantic population. However, selected statistical methods are quite robust to slight deviations from normality when homogeneity of variances is achieved (as it is the case, see 2.2.2.3).

```
#Large MHW
```

```
Pmax_Stats_LMHW_NO %>%  
  group_by(Population, Heat_Treatment, Phase) %>%  
  shapiro_test(Value_Log)
```

```
## # A tibble: 12 x 6  
##   Phase      Population Heat_Treatment variable statistic      p  
##   <fct>      <fct>      <fct>      <chr>      <dbl> <dbl>  
## 1 MHW        Cantabria   Control    Value_Log    0.981 0.955  
## 2 Recovery  Cantabria   Control    Value_Log    0.889 0.314  
## 3 MHW        Cantabria   MHW2       Value_Log    0.979 0.947  
## 4 Recovery  Cantabria   MHW2       Value_Log    0.929 0.574  
## 5 MHW        Cantabria   MHW4       Value_Log    0.843 0.137  
## 6 Recovery  Cantabria   MHW4       Value_Log    0.910 0.433  
## 7 MHW        Portugal    Control    Value_Log    0.831 0.171  
## 8 Recovery  Portugal    Control    Value_Log    0.970 0.839  
## 9 MHW        Portugal    MHW2       Value_Log    0.886 0.299  
## 10 Recovery Portugal    MHW2       Value_Log    0.917 0.486  
## 11 MHW        Portugal    MHW4       Value_Log    0.854 0.208  
## 12 Recovery Portugal    MHW4       Value_Log    0.841 0.168
```

```
ggqqplot(Pmax_Stats_LMHW_NO, "Value_Log", ggtheme = theme_bw()) +  
  facet_grid(Population ~ Heat_Treatment ~ Phase, labeller = "label_both")
```



```
#Short MHW
```

```
Pmax_Stats_SMHW_NO %>%
```

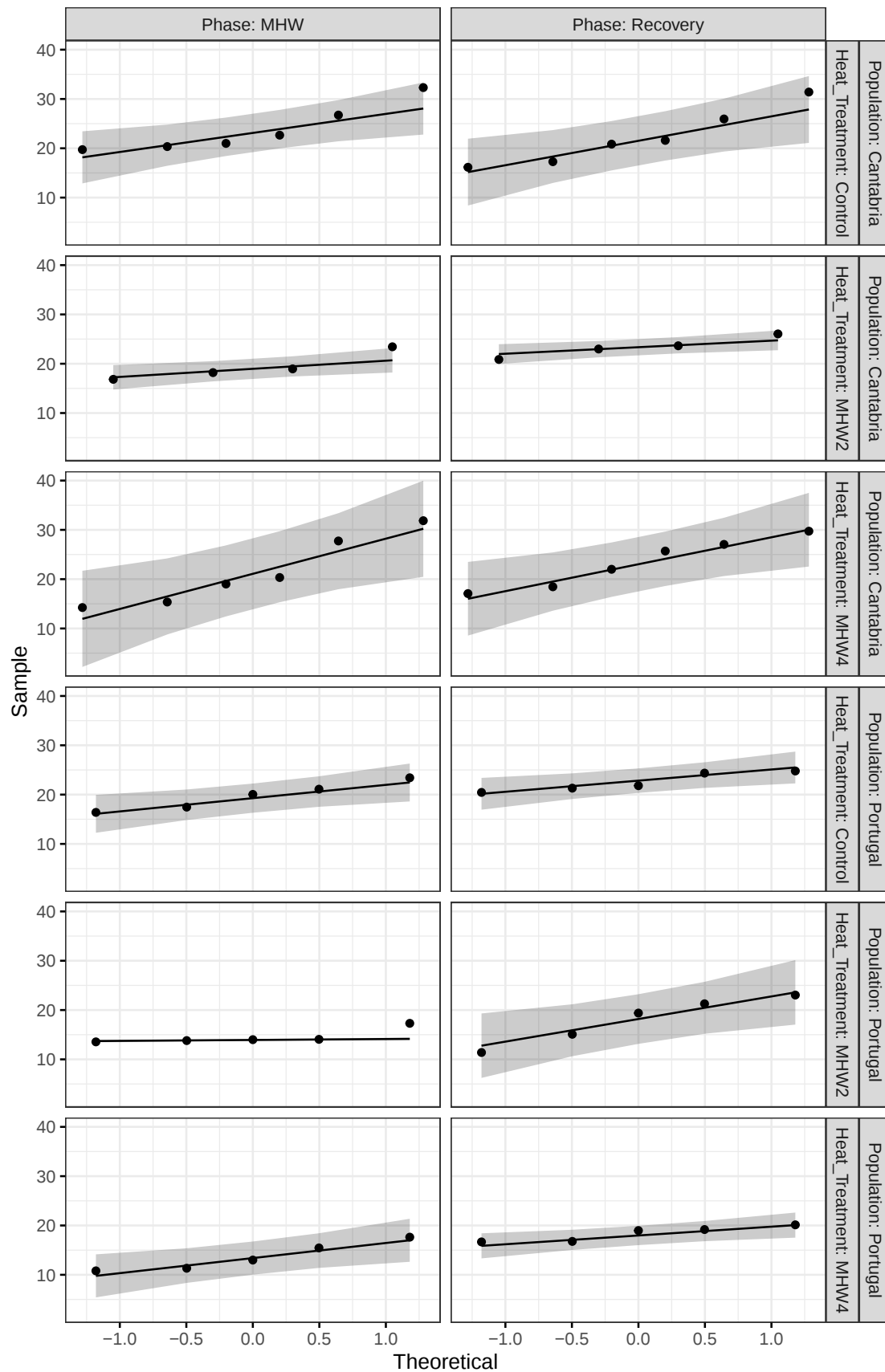
```
  group_by(Population, Heat_Treatment, Phase) %>%
```

```
  shapiro_test(Value)
```

```
## # A tibble: 12 x 6
```

##	Phase	Population	Heat_Treatment	variable	statistic	p
##	<fct>	<fct>	<fct>	<chr>	<dbl>	<dbl>
## 1	MHW	Cantabria	Control	Value	0.847	0.150
## 2	Recovery	Cantabria	Control	Value	0.937	0.631
## 3	MHW	Cantabria	MHW2	Value	0.888	0.372
## 4	Recovery	Cantabria	MHW2	Value	0.986	0.937
## 5	MHW	Cantabria	MHW4	Value	0.912	0.448
## 6	Recovery	Cantabria	MHW4	Value	0.946	0.705
## 7	MHW	Portugal	Control	Value	0.966	0.852
## 8	Recovery	Portugal	Control	Value	0.887	0.342
## 9	MHW	Portugal	MHW2	Value	0.676	0.00522
## 10	Recovery	Portugal	MHW2	Value	0.946	0.707
## 11	MHW	Portugal	MHW4	Value	0.927	0.578
## 12	Recovery	Portugal	MHW4	Value	0.869	0.262

```
ggqqplot(Pmax_Stats_SMHW_NO, "Value", ggtheme = theme_bw()) +  
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")
```



2.2.2.3. Homogeneity of variances

Homogeneity of variances was checked through Levene's tests. Tests using the complete dataset did not reveal heteroscedasticity ($p > 0.05$) for any MHW duration (Large/Short).

```
#Large MHW
```

```
Pmax_Stats_LMHW_NO %>%  
  levene_test(Value_Log ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4  
##   df1 df2 statistic p  
##   <int> <int>   <dbl> <dbl>  
## 1    11    54     1.99 0.0477
```

```
Pmax_Stats_LMHW_NO %>%  
  group_by(Phase) %>%  
  levene_test(Value_Log ~ Heat_Treatment*Population)
```

```
## # A tibble: 2 x 5  
##   Phase df1 df2 statistic p  
##   <fct> <int> <int>   <dbl> <dbl>  
## 1 MHW      5    27     1.40 0.255  
## 2 Recovery  5    27     2.36 0.0670
```

```
Pmax_Stats_LMHW_NO %>%  
  group_by(Phase, Population) %>%  
  levene_test(Value_Log ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6  
##   Phase Population df1 df2 statistic p  
##   <fct>   <fct>   <int> <int>   <dbl> <dbl>  
## 1 MHW      Cantabria  2    15     0.616 0.553  
## 2 MHW      Portugal    2    12     1.92 0.189  
## 3 Recovery Cantabria  2    15     2.50 0.116  
## 4 Recovery Portugal    2    12     3.91 0.0493
```

```
#Short MHW
```

```
Pmax_Stats_SMHW_NO %>%  
  levene_test(Value ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4  
##   df1 df2 statistic p  
##   <int> <int>   <dbl> <dbl>  
## 1    11    50     1.53 0.151
```

```
Pmax_Stats_SMHW_NO %>%  
  group_by(Phase) %>%  
  levene_test(Value ~ Heat_Treatment*Population)
```

```
## # A tibble: 2 x 5  
##   Phase df1 df2 statistic p  
##   <fct> <int> <int>   <dbl> <dbl>  
## 1 MHW      5    25     1.54 0.215  
## 2 Recovery  5    25     1.90 0.131
```

```
Pmax_Stats_SMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(Value ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Phase      Population    df1    df2 statistic      p
##   <fct>      <fct>      <int> <int>    <dbl> <dbl>
## 1 MHW        Cantabria         2     13     0.996 0.396
## 2 MHW        Portugal          2     12     1.28 0.314
## 3 Recovery Cantabria         2     13     1.48 0.263
## 4 Recovery Portugal          2     12     2.17 0.157
```

2.2.3. Mixed model

From the model, we observed that there was not a statistically significant ($p > .05$) three-way interaction between time, population, and MHW intensity for any MHW duration. For the Long MHW, significant two-way interactions were not detected. Any factor seemed to have effects over Pmax. For the Short MHW, significant interactions were not detected, but it seems that individual effects of population ($F[1, 22]=9.79$, $p=0.005$) and phase ($F[1, 25]=21.356$, $p<0.001$) were significant.

```
#LRec
lmer_LRec_Pmax <- lmerTest::lmer(Value_Log ~ Population*Heat_Treatment*Phase
                                + (1 | Tank) + (1 | ID),
                                Pmax_Stats_LMHW_NO, REML = TRUE)
summary(lmer_LRec_Pmax, ddf="Kenward-Roger") #LRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: Value_Log ~ Population * Heat_Treatment * Phase + (1 | Tank) +
##         (1 | ID)
## Data: Pmax_Stats_LMHW_NO
##
## REML criterion at convergence: -115.3
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.42041 -0.51434  0.03395  0.47427  1.70062
##
## Random effects:
##  Groups   Name                Variance Std.Dev.
##  ID       (Intercept) 3.112e-03 5.578e-02
##  Tank     (Intercept) 9.196e-12 3.033e-06
##  Residual                    2.569e-03 5.068e-02
## Number of obs: 66, groups: ID, 33; Tank, 6
##
## Fixed effects:
##
##              Estimate Std. Error
## (Intercept)    1.370205   0.030768
## PopulationPortugal -0.013833   0.048649
## Heat_TreatmentMHW2  0.006244   0.043513
## Heat_TreatmentMHW4  0.080663   0.043513
## PhaseRecovery    -0.015480   0.029260
```

```

## PopulationPortugal:Heat_TreatmentMHW2          -0.014717    0.065269
## PopulationPortugal:Heat_TreatmentMHW4          -0.035372    0.066881
## PopulationPortugal:PhaseRecovery                0.030589    0.046265
## Heat_TreatmentMHW2:PhaseRecovery               0.042134    0.041380
## Heat_TreatmentMHW4:PhaseRecovery               -0.056020    0.041380
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery -0.055107    0.062071
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 0.083062    0.063435
##                                                    df t value Pr(>|t|)
## (Intercept)                                14.964078  44.533 <2e-16
## PopulationPortugal                        37.270186  -0.284  0.7777
## Heat_TreatmentMHW2                       14.964078   0.143  0.8878
## Heat_TreatmentMHW4                       14.964078   1.854  0.0836
## PhaseRecovery                           27.000000  -0.529  0.6011
## PopulationPortugal:Heat_TreatmentMHW2       37.270186  -0.225  0.8228
## PopulationPortugal:Heat_TreatmentMHW4       37.587863  -0.529  0.6000
## PopulationPortugal:PhaseRecovery            27.000000   0.661  0.5141
## Heat_TreatmentMHW2:PhaseRecovery            27.000000   1.018  0.3176
## Heat_TreatmentMHW4:PhaseRecovery            27.000000  -1.354  0.1870
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 27.000000  -0.888  0.3825
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 27.000000   1.309  0.2014
##
## (Intercept)                                ***
## PopulationPortugal
## Heat_TreatmentMHW2
## Heat_TreatmentMHW4
## PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW2
## PopulationPortugal:Heat_TreatmentMHW4
## PopulationPortugal:PhaseRecovery
## Heat_TreatmentMHW2:PhaseRecovery
## Heat_TreatmentMHW4:PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PopltnPrtgl -0.632
## Ht_TrtnMHW2 -0.707  0.447
## Ht_TrtnMHW4 -0.707  0.447  0.500
## PhaseRecvry -0.475  0.301  0.336   0.336
## PpP:H_TMHW2  0.471 -0.745 -0.667  -0.333  -0.224
## PpP:H_TMHW4  0.461 -0.729 -0.326  -0.652  -0.219  0.544
## PpltnPrt:PR  0.301 -0.475 -0.213  -0.213  -0.632  0.354   0.347
## Ht_TMHW2:PR  0.336 -0.213 -0.475  -0.238  -0.707  0.317   0.155
## Ht_TMHW4:PR  0.336 -0.213 -0.238  -0.475  -0.707  0.158   0.310
## PP:H_TMHW2: -0.224  0.354  0.317   0.158   0.471 -0.475  -0.258
## PP:H_TMHW4: -0.219  0.347  0.155   0.310   0.461 -0.258  -0.475
##      PpP:PR H_TMHW2: H_TMHW4: PP:H_TMHW2:
## PopltnPrtgl
## Ht_TrtnMHW2
## Ht_TrtnMHW4
## PhaseRecvry

```

```
## PpP:H_TMHW2
## PpP:H_TMHW4
## PpltnPrt:PR
## Ht_TMHW2:PR 0.447
## Ht_TMHW4:PR 0.447 0.500
## PP:H_TMHW2: -0.745 -0.667 -0.333
## PP:H_TMHW4: -0.729 -0.326 -0.652 0.544
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
anova(lmer_LRec_Pmax, type = 3, ddf="Kenward-Roger") #LRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##
```

	Sum Sq	Mean Sq	NumDF	DenDF	F value
Population	0.0005242	0.0005242	1	24.1720	0.2041
Heat_Treatment	0.0115917	0.0057959	2	3.0646	2.2515
Phase	0.0000004	0.0000004	1	27.0000	0.0002
Population:Heat_Treatment	0.0023133	0.0011567	2	24.1725	0.4503
Population:Phase	0.0064179	0.0064179	1	27.0000	2.4987
Heat_Treatment:Phase	0.0024163	0.0012082	2	27.0000	0.4704
Population:Heat_Treatment:Phase	0.0137328	0.0068664	2	27.0000	2.6733

```
## Pr(>F)
## Population 0.65547
## Heat_Treatment 0.25029
## Phase 0.99028
## Population:Heat_Treatment 0.64266
## Population:Phase 0.12559
## Heat_Treatment:Phase 0.62979
## Population:Heat_Treatment:Phase 0.08724 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#SRec
```

```
lmer_SRec_Pmax <- lmerTest::lmer(Value ~ Population*Heat_Treatment*Phase
+ (1 | Tank) + (1 | ID),
Pmax_Stats_SMHW_NO, REML = TRUE)
summary(lmer_SRec_Pmax, ddf="Kenward-Roger") #SRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: Value ~ Population * Heat_Treatment * Phase + (1 | Tank) + (1 |
## ID)
## Data: Pmax_Stats_SMHW_NO
##
## REML criterion at convergence: 285.5
##
## Scaled residuals:
## Min 1Q Median 3Q Max
## -1.65416 -0.53252 0.03638 0.46684 1.53194
##
## Random effects:
## Groups Name Variance Std.Dev.
## ID (Intercept) 1.285e+01 3.584e+00
```

```

## Tank      (Intercept) 8.094e-10 2.845e-05
## Residual      4.696e+00 2.167e+00
## Number of obs: 62, groups: ID, 31; Tank, 6
##
## Fixed effects:
##
##                                     Estimate Std. Error    df
## (Intercept)                    23.7970      1.7098 10.7014
## PopulationPortugal              -4.1165      2.5549 29.3400
## Heat_TreatmentMHW2             -4.4403      2.7034 15.1683
## Heat_TreatmentMHW4             -2.3703      2.4180 10.7014
## PhaseRecovery                  -1.5914      1.2511 25.0000
## PopulationPortugal:Heat_TreatmentMHW2 -0.6987      3.8077 29.2817
## PopulationPortugal:Heat_TreatmentMHW4 -3.6711      3.6132 29.3400
## PopulationPortugal:PhaseRecovery      4.4525      1.8556 25.0000
## Heat_TreatmentMHW2:PhaseRecovery      5.6221      1.9781 25.0000
## Heat_TreatmentMHW4:PhaseRecovery      3.5006      1.7693 25.0000
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery -4.9913      2.7694 25.0000
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery -1.6580      2.6243 25.0000
##
##                                     t value Pr(>|t|)
## (Intercept)                    13.918 3.41e-08 ***
## PopulationPortugal             -1.611 0.11784
## Heat_TreatmentMHW2            -1.642 0.12106
## Heat_TreatmentMHW4            -0.980 0.34861
## PhaseRecovery                  -1.272 0.21507
## PopulationPortugal:Heat_TreatmentMHW2 -0.183 0.85568
## PopulationPortugal:Heat_TreatmentMHW4 -1.016 0.31792
## PopulationPortugal:PhaseRecovery      2.399 0.02419 *
## Heat_TreatmentMHW2:PhaseRecovery      2.842 0.00879 **
## Heat_TreatmentMHW4:PhaseRecovery      1.979 0.05898 .
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery -1.802 0.08356 .
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery -0.632 0.53325
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PopltnPrtgl -0.674
## Ht_TrtmMHW2 -0.632 0.426
## Ht_TrtmMHW4 -0.707 0.477 0.447
## PhaseRecvry -0.366 0.247 0.231 0.259
## PpP:H_TMHW2 0.452 -0.670 -0.714 -0.319 -0.165
## PpP:H_TMHW4 0.477 -0.707 -0.302 -0.674 -0.174 0.474
## PpltnPrt:PR 0.247 -0.366 -0.156 -0.174 -0.674 0.245 0.259
## Ht_TMHW2:PR 0.231 -0.156 -0.366 -0.164 -0.632 0.261 0.110
## Ht_TMHW4:PR 0.259 -0.174 -0.164 -0.366 -0.707 0.117 0.247
## PP:H_TMHW2: -0.165 0.245 0.261 0.117 0.452 -0.366 -0.173
## PP:H_TMHW4: -0.174 0.259 0.110 0.247 0.477 -0.173 -0.366
##
##      PpP:PR H_TMHW2: H_TMHW4: PP:H_TMHW2:
## PopltnPrtgl
## Ht_TrtmMHW2
## Ht_TrtmMHW4
## PhaseRecvry
## PpP:H_TMHW2
## PpP:H_TMHW4

```

```
## PpltnPrt:PR
## Ht_TMH2:PR 0.426
## Ht_TMH4:PR 0.477 0.447
## PP:H_TMH2: -0.670 -0.714 -0.319
## PP:H_TMH4: -0.707 -0.302 -0.674 0.474
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
anova(lmer_SRec_Pmax, type = 3, ddf="Kenward-Roger") #SRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##
## Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Population 45.964 45.964 1 22.4527 9.7889 0.004806
## Heat_Treatment 19.834 9.917 2 2.9204 2.1054 0.271548
## Phase 100.279 100.279 1 25.0000 21.3561 9.945e-05
## Population:Heat_Treatment 8.820 4.410 2 22.4547 0.9392 0.405777
## Population:Phase 19.015 19.015 1 25.0000 4.0495 0.055077
## Heat_Treatment:Phase 29.591 14.796 2 25.0000 3.1510 0.060202
## Population:Heat_Treatment:Phase 15.552 7.776 2 25.0000 1.6560 0.211159
##
## Population **
## Heat_Treatment
## Phase ***
## Population:Heat_Treatment
## Population:Phase .
## Heat_Treatment:Phase .
## Population:Heat_Treatment:Phase
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.2.4. Post-HOC analysis

For the Large MHW:

Not computed as no effects were detected for any factor.

For the Short MHW:

1. Simple two-way interaction analysis: It was not computed considering that there was not significant three-way interactions among treatments.
2. Simple-simple main effect analysis: It was not computed considering that there was not significant two-way interactions among treatments.
3. Simple comparisons: No significant differences.

```
pwc_SRec_Pmax <- emmeans(lmer_SRec_Pmax, ~ Heat_Treatment | Phase * Population) %>%
  pairs(adjust="Tukey")
pwc_SRec_Pmax
```

```
## Phase = MHW, Population = Cantabria:
## contrast estimate SE df t.ratio p.value
## Control - MHW2 4.4403 2.70 15.2 1.642 0.2588
```

```
## Control - MHW4    2.3703 2.42 10.7    0.980 0.6040
## MHW2 - MHW4      -2.0701 2.70 15.2   -0.766 0.7290
##
## Phase = Recovery, Population = Cantabria:
## contrast      estimate    SE    df t.ratio p.value
## Control - MHW2  -1.1817 2.70 15.2   -0.437 0.9007
## Control - MHW4  -1.1304 2.42 10.7   -0.467 0.8877
## MHW2 - MHW4      0.0514 2.70 15.2    0.019 0.9998
##
## Phase = MHW, Population = Portugal:
## contrast      estimate    SE    df t.ratio p.value
## Control - MHW2   5.1390 2.68 13.5    1.916 0.1720
## Control - MHW4   6.0414 2.68 13.5    2.250 0.0983
## MHW2 - MHW4      0.9024 2.68 13.5    0.337 0.9398
##
## Phase = Recovery, Population = Portugal:
## contrast      estimate    SE    df t.ratio p.value
## Control - MHW2   4.5083 2.68 13.5    1.681 0.2478
## Control - MHW4   4.1987 2.68 13.5    1.564 0.2941
## MHW2 - MHW4     -0.3095 2.68 13.5   -0.115 0.9927
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

2.3. Respiration rate analysis

2.3.1. Prepare data

```
Resp_Stats <- PI[PI$Parameter == "Resp",]
Resp_Stats$ID <- as.factor(Resp_Stats$ID)
Resp_Stats$Phase <- as.factor(Resp_Stats$Phase)
Resp_Stats$Phase <- as.factor(Resp_Stats$Phase)
Resp_Stats$Tank <- as.factor(Resp_Stats$Tank)
Resp_Stats$Heat_Treatment <- as.factor(Resp_Stats$Heat_Treatment)
Resp_Stats$Population <- as.factor(Resp_Stats$Population)
Resp_Stats$Value_Log <- log10(Resp_Stats$Value + 1)
summary(Resp_Stats)
```

```
##      Day      Phase  Duration_Treatment  Population
## Length:144      MHW      :72  Length:144      Cantabria:72
## Class :character Recovery:72  Class :character  Portugal :72
## Mode  :character              Mode  :character
##
##
##
## Heat_Treatment  Sensor      ID      Parameter
## Control:48      Length:144    1      : 4  Length:144
## MHW2   :48      Class :character 2      : 4  Class :character
## MHW4   :48      Mode  :character 3      : 4  Mode  :character
##
##
##      4      : 4
##      5      : 4
```

```
##              6      : 4
##              (Other):120
##      Value      Tank      Value_Log
##  Min.   :0.7714   1       :12   Min.   :0.2483
## 1st Qu.:2.9871   2       :12   1st Qu.:0.6007
## Median :3.8683   3       :12   Median :0.6874
## Mean   :4.0115   4       :12   Mean   :0.6772
## 3rd Qu.:4.7593   5       :12   3rd Qu.:0.7604
## Max.   :9.8494   6       :12   Max.   :1.0354
##              (Other):72
```

```
Resp_Stats_SMHW <- Resp_Stats[Resp_Stats$Duration_Treatment == "Srec",]
Resp_Stats_LMHW <- Resp_Stats[Resp_Stats$Duration_Treatment == "Lrec",]
```

2.3.2. Check Model assumptions

2.3.2.1. Outliers

First of all, the outliers were identified and removed when considered extreme.

```
# Large MHW
outliers_LMHW_Resp <- Resp_Stats_LMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Value)
outliers_LMHW_Resp <- outliers_LMHW_Resp[-c(11,12)]
Resp_Stats_LMHW_NO <- anti_join(Resp_Stats_LMHW, outliers_LMHW_Resp, by = c("ID"))
outliers_LMHW_Resp %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, Value) %>%
  head() # Large MHW outliers
```

```
## # A tibble: 4 x 7
##   Phase      Population Heat_Treatment Day   Duration_Treatment ID      Value
##   <fct>      <fct>      <fct>      <chr> <chr>              <fct> <dbl>
## 1 MHW        Cantabria   Control    21    Lrec                1      5.59
## 2 MHW        Cantabria   MHW4       21    Lrec                10     3.18
## 3 MHW        Portugal    MHW2       21    Lrec                31     5.40
## 4 Recovery Portugal    MHW2       28    Lrec                31     9.24
```

```
# Short MHW
outliers_SMHW_Resp <- Resp_Stats_SMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Value)
outliers_SMHW_Resp <- outliers_SMHW_Resp[-c(11,12)]
Resp_Stats_SMHW_NO <- anti_join(Resp_Stats_SMHW, outliers_SMHW_Resp, by = c("ID"))
outliers_SMHW_Resp %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, Value) %>%
  head() # Short MHW outliers
```

```
## # A tibble: 4 x 7
##   Phase      Population Heat_Treatment Day   Duration_Treatment ID      Value
##   <fct>      <fct>      <fct>      <chr> <chr>              <fct> <dbl>
## 1 MHW        Cantabria   Control    7     Srec                5      4.27
```

## 2	Recovery	Cantabria	Control	14	Srec	5	4.33
## 3	Recovery	Cantabria	MHW2	14	Srec	17	2.28
## 4	Recovery	Cantabria	MHW4	14	Srec	11	3.30

2.3.2.2. Normality

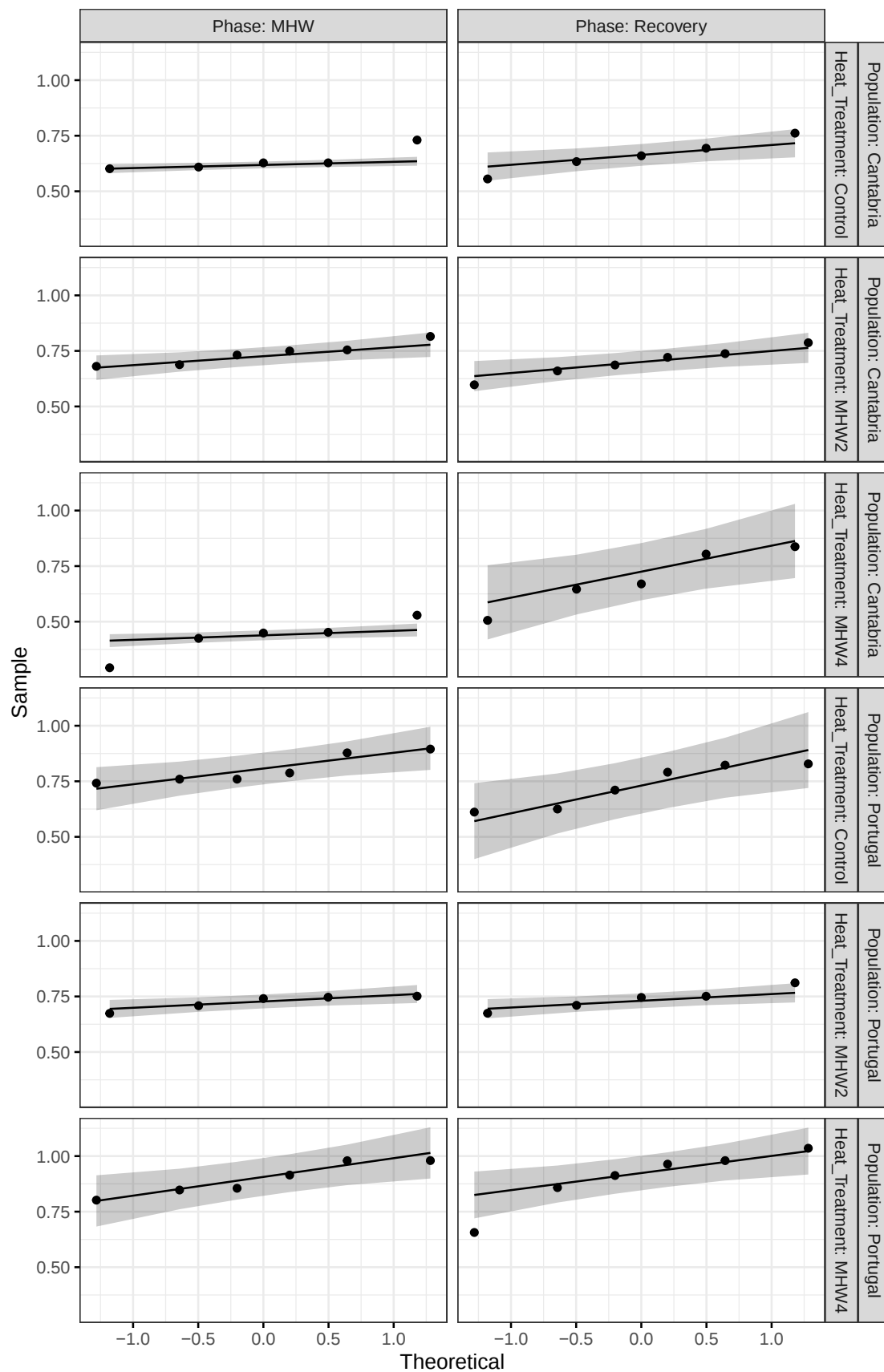
The second step was to test normality. For that purpose, we computed Shapiro-Wilk's tests for each combinations of factor levels. Normality was also assessed visually by inspecting qqplots. According to the results, no clear deviations from normality were observed, with the exception of Large MHW/Cantabria/Control/After MHW. We decided not to transform data as the selected statistical methods are quite robust to slight deviations from normality when homogeneity of variances is achieved (see 2.2.2.3).

```
#Large MHW
```

```
Resp_Stats_LMHW_NO %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  shapiro_test(Value_Log)
```

```
## # A tibble: 12 x 6
##   Phase      Population Heat_Treatment variable statistic      p
##   <fct>      <fct>      <fct>      <chr>      <dbl> <dbl>
## 1 MHW        Cantabria   Control    Value_Log    0.746 0.0276
## 2 Recovery   Cantabria   Control    Value_Log    0.995 0.993
## 3 MHW        Cantabria   MHW2       Value_Log    0.936 0.629
## 4 Recovery   Cantabria   MHW2       Value_Log    0.991 0.992
## 5 MHW        Cantabria   MHW4       Value_Log    0.907 0.450
## 6 Recovery   Cantabria   MHW4       Value_Log    0.942 0.681
## 7 MHW        Portugal    Control    Value_Log    0.825 0.0974
## 8 Recovery   Portugal    Control    Value_Log    0.861 0.192
## 9 MHW        Portugal    MHW2       Value_Log    0.861 0.230
## 10 Recovery  Portugal    MHW2       Value_Log    0.976 0.912
## 11 MHW        Portugal    MHW4       Value_Log    0.903 0.390
## 12 Recovery  Portugal    MHW4       Value_Log    0.885 0.295
```

```
ggqqplot(Resp_Stats_LMHW_NO, "Value_Log", ggtheme = theme_bw()) +
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")
```



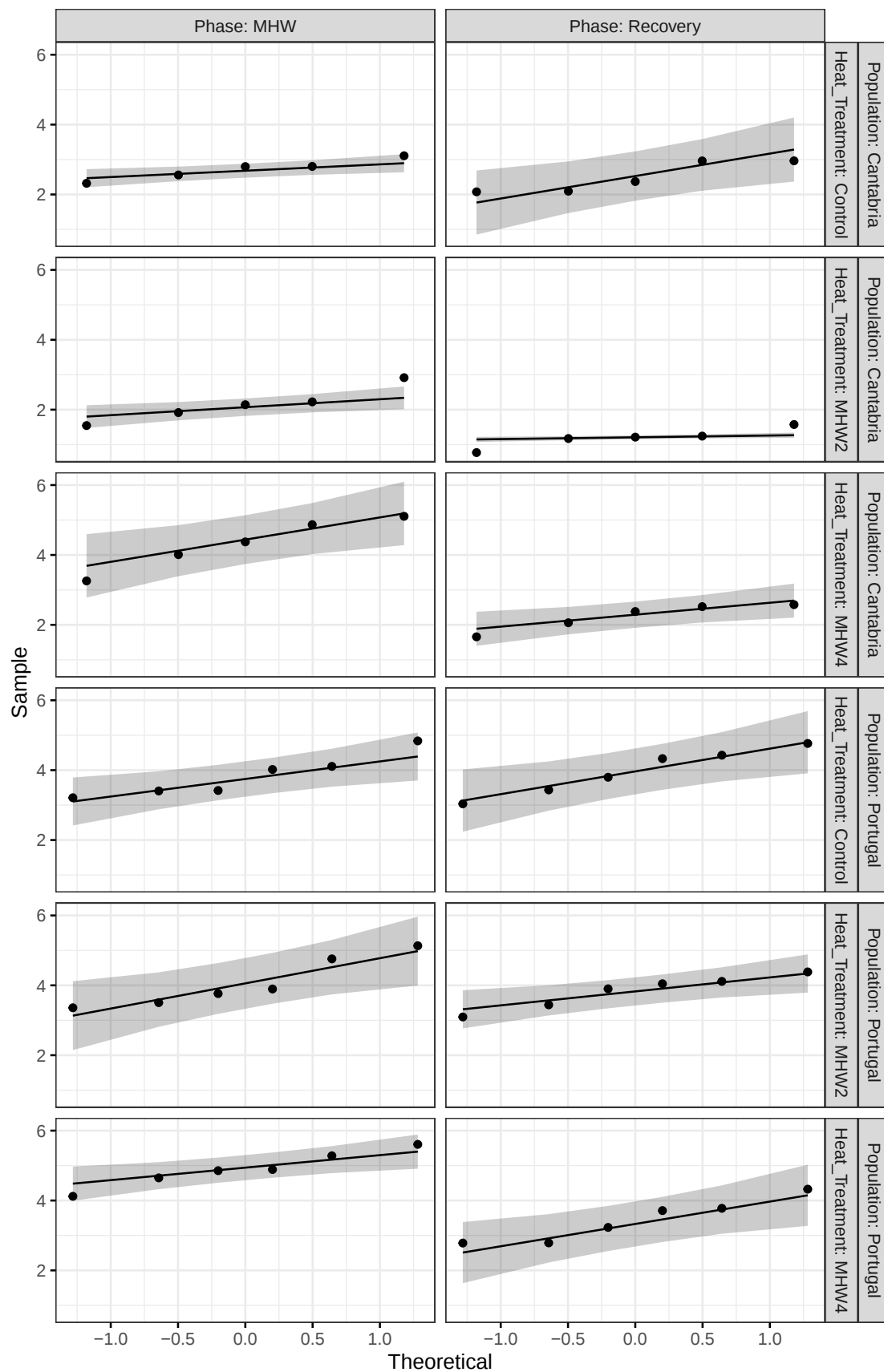
```

# Short MHW
Resp_Stats_SMHw_NO %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  shapiro_test(Value)

## # A tibble: 12 x 6
##   Phase      Population Heat_Treatment variable statistic      p
##   <fct>      <fct>      <fct>      <chr>      <dbl> <dbl>
## 1 MHW        Cantabria  Control      Value        0.971 0.880
## 2 Recovery  Cantabria  Control      Value        0.810 0.0973
## 3 MHW        Cantabria  MHW2         Value        0.959 0.798
## 4 Recovery  Cantabria  MHW2         Value        0.928 0.581
## 5 MHW        Cantabria  MHW4         Value        0.961 0.812
## 6 Recovery  Cantabria  MHW4         Value        0.895 0.381
## 7 MHW        Portugal   Control      Value        0.903 0.391
## 8 Recovery  Portugal   Control      Value        0.956 0.792
## 9 MHW        Portugal   MHW2         Value        0.887 0.301
## 10 Recovery Portugal   MHW2         Value        0.940 0.655
## 11 MHW        Portugal   MHW4         Value        0.980 0.949
## 12 Recovery Portugal   MHW4         Value        0.919 0.500

ggqqplot(Resp_Stats_SMHw_NO, "Value", ggtheme = theme_bw()) +
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")

```



2.3.2.3. Homogeneity of variances

Homogeneity of variances was checked through Levene's tests. Tests using the complete dataset did not reveal heteroscedasticity ($p > 0.05$) for any MHW duration (Large/Short).

```
#Large MHW
```

```
Resp_Stats_LMHW_NO %>%  
  levene_test(Value_Log ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4  
##   df1 df2 statistic p  
##   <int> <int>   <dbl> <dbl>  
## 1    11    54     1.12 0.361
```

```
Resp_Stats_LMHW_NO %>%  
  group_by(Phase) %>%  
  levene_test(Value_Log ~ Heat_Treatment*Population)
```

```
## # A tibble: 2 x 5  
##   Phase df1 df2 statistic p  
##   <fct> <int> <int>   <dbl> <dbl>  
## 1 MHW      5    27    0.648 0.665  
## 2 Recovery  5    27    0.921 0.483
```

```
Resp_Stats_LMHW_NO %>%  
  group_by(Phase, Population) %>%  
  levene_test(Value_Log ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6  
##   Phase Population df1 df2 statistic p  
##   <fct>   <fct>   <int> <int>   <dbl> <dbl>  
## 1 MHW      Cantabria  2    13    0.320 0.732  
## 2 MHW      Portugal   2    14    1.41  0.276  
## 3 Recovery Cantabria  2    13    1.17  0.342  
## 4 Recovery Portugal   2    14    1.12  0.355
```

```
#Short MHW
```

```
Resp_Stats_SMHW_NO %>%  
  levene_test(Value ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4  
##   df1 df2 statistic p  
##   <int> <int>   <dbl> <dbl>  
## 1    11    54    0.867 0.576
```

```
Resp_Stats_SMHW_NO %>%  
  group_by(Phase) %>%  
  levene_test(Value ~ Heat_Treatment*Population)
```

```
## # A tibble: 2 x 5  
##   Phase df1 df2 statistic p  
##   <fct> <int> <int>   <dbl> <dbl>  
## 1 MHW      5    27    0.680 0.643  
## 2 Recovery  5    27    1.37  0.267
```

```
Resp_Stats_SMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(Value ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Phase      Population    df1    df2 statistic      p
##   <fct>      <fct>      <int> <int>     <dbl> <dbl>
## 1 MHW        Cantabria         2     12      1.30  0.308
## 2 MHW        Portugal          2     15      0.304  0.742
## 3 Recovery Cantabria         2     12      0.650  0.540
## 4 Recovery Portugal          2     15      0.691  0.516
```

2.3.3. Mixed model

For the long MHW, there was a significant ($p < .05$) three-way interaction between time, population, and MHW intensity ($F[2, 27] = 7.154$, $p = 0.003$). From the model for the Short MHW what we observed was that there was not a statistically significant ($p > .05$) three-way interaction between time, population, and MHW intensity ($F[2, 27] = 0.239$, $p = 0.789$), but there was a significant two-way interactions between Time/MHW intensity ($F[2, 27] = 21.582$, $p < 0.001$), Population/MHW intensity ($F[2, 24] = 6.687$, $p = 0.004$) and Population/Time ($F[2, 27] = 6.601$, $p = 0.016$)

```
#LRec
lmer_LRec_Resp <- lmerTest::lmer(Value_Log ~ Population*Heat_Treatment*Phase
                                + (1 | Tank) + (1 | ID),
                                Resp_Stats_LMHW_NO, REML = TRUE)
summary(lmer_LRec_Resp, ddf="Kenward-Roger") #SRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: Value_Log ~ Population * Heat_Treatment * Phase + (1 | Tank) +
##          (1 | ID)
## Data: Resp_Stats_LMHW_NO
##
## REML criterion at convergence: -98.5
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.37640 -0.36592 -0.02159  0.55136  1.44883
##
## Random effects:
##   Groups   Name                Variance Std.Dev.
##   ID       (Intercept) 0.002224 0.04715
##   Tank     (Intercept) 0.000000 0.00000
##   Residual                    0.004619 0.06796
## Number of obs: 66, groups: ID, 33; Tank, 6
##
## Fixed effects:
##                                     Estimate Std. Error
## (Intercept)                        0.63940    0.03733
## PopulationPortugal                  0.16406    0.05034
## Heat_TreatmentMHW2                  0.09715    0.05034
## Heat_TreatmentMHW4                 -0.20994    0.05280
```

```

## PhaseRecovery 0.02139 0.04298
## PopulationPortugal:Heat_TreatmentMHW2 -0.17641 0.07119
## PopulationPortugal:Heat_TreatmentMHW4 0.30288 0.07119
## PopulationPortugal:PhaseRecovery -0.09341 0.05820
## Heat_TreatmentMHW2:PhaseRecovery -0.05962 0.05820
## Heat_TreatmentMHW4:PhaseRecovery 0.24177 0.06079
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 0.14597 0.08231
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery -0.16515 0.08231
## df t value Pr(>|t|)
## (Intercept) 23.22869 17.127 1.13e-14
## PopulationPortugal 45.14004 3.259 0.002128
## Heat_TreatmentMHW2 21.12310 1.930 0.067161
## Heat_TreatmentMHW4 23.22869 -3.976 0.000588
## PhaseRecovery 27.00000 0.498 0.622716
## PopulationPortugal:Heat_TreatmentMHW2 45.14004 -2.478 0.017020
## PopulationPortugal:Heat_TreatmentMHW4 45.14004 4.255 0.000104
## PopulationPortugal:PhaseRecovery 27.00000 -1.605 0.120147
## Heat_TreatmentMHW2:PhaseRecovery 27.00000 -1.024 0.314701
## Heat_TreatmentMHW4:PhaseRecovery 27.00000 3.977 0.000470
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 27.00000 1.774 0.087421
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 27.00000 -2.007 0.054911
##
## (Intercept) ***
## PopulationPortugal **
## Heat_TreatmentMHW2 .
## Heat_TreatmentMHW4 ***
## PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW2 *
## PopulationPortugal:Heat_TreatmentMHW4 ***
## PopulationPortugal:PhaseRecovery
## Heat_TreatmentMHW2:PhaseRecovery
## Heat_TreatmentMHW4:PhaseRecovery ***
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery .
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
## (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PopltnPrtgl -0.739
## Ht_TrtnMHW2 -0.739 0.545
## Ht_TrtnMHW4 -0.707 0.522 0.522
## PhaseRecvry -0.581 0.429 0.429 0.411
## PpP:H_TMHW2 0.522 -0.707 -0.707 -0.369 -0.303
## PpP:H_TMHW4 0.522 -0.707 -0.386 -0.739 -0.303 0.500
## PpltnPrt:PR 0.429 -0.581 -0.317 -0.303 -0.739 0.411 0.411
## Ht_TMHW2:PR 0.429 -0.317 -0.581 -0.303 -0.739 0.411 0.224
## Ht_TMHW4:PR 0.411 -0.303 -0.303 -0.581 -0.707 0.215 0.429
## PP:H_TMHW2: -0.303 0.411 0.411 0.215 0.522 -0.581 -0.290
## PP:H_TMHW4: -0.303 0.411 0.224 0.429 0.522 -0.290 -0.581
## PpP:PR H_TMHW2: H_TMHW4: PP:H_TMHW2:
## PopltnPrtgl
## Ht_TrtnMHW2
## Ht_TrtnMHW4

```

```
## PhaseRecvry
## PpP:H_TMHW2
## PpP:H_TMHW4
## PpltnPrt:PR
## Ht_TMHW2:PR 0.545
## Ht_TMHW4:PR 0.522 0.522
## PP:H_TMHW2: -0.707 -0.707 -0.369
## PP:H_TMHW4: -0.707 -0.386 -0.739 0.500
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
anova(lmer_LRec_Resp, type = 3, ddf="Kenward-Roger") #LRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##
```

	Sum Sq	Mean Sq	NumDF	DenDF	F value
Population	0.200679	0.200679	1	24.4719	43.4471
Heat_Treatment	0.002626	0.001313	2	2.8822	0.2843
Phase	0.016977	0.016977	1	27.0000	3.6756
Population:Heat_Treatment	0.149680	0.074840	2	24.4719	16.2029
Population:Phase	0.040744	0.040744	1	27.0000	8.8210
Heat_Treatment:Phase	0.085072	0.042536	2	27.0000	9.2091
Population:Heat_Treatment:Phase	0.066083	0.033041	2	27.0000	7.1535

```
## Pr(>F)
## Population 7.360e-07 ***
## Heat_Treatment 0.7714525
## Phase 0.0658519 .
## Population:Heat_Treatment 3.298e-05 ***
## Population:Phase 0.0061830 **
## Heat_Treatment:Phase 0.0008929 ***
## Population:Heat_Treatment:Phase 0.0032144 **
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#SRec
```

```
lmer_SRec_Resp <- lmerTest::lmer(Value ~ Population*Heat_Treatment*Phase
+ (1 | Tank) + (1 | ID),
Resp_Stats_SMHW_NO, REML = TRUE)
summary(lmer_SRec_Resp, ddf="Kenward-Roger") #SRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: Value ~ Population * Heat_Treatment * Phase + (1 | Tank) + (1 |
## ID)
## Data: Resp_Stats_SMHW_NO
##
## REML criterion at convergence: 105
##
## Scaled residuals:
## Min 1Q Median 3Q Max
## -1.49487 -0.57339 -0.03999 0.55738 1.74993
##
## Random effects:
## Groups Name Variance Std.Dev.
```

```

## ID      (Intercept) 1.014e-01 3.184e-01
## Tank    (Intercept) 6.373e-18 2.524e-09
## Residual      1.969e-01 4.437e-01
## Number of obs: 66, groups: ID, 33; Tank, 6
##
## Fixed effects:
##
##                                     Estimate Std. Error    df
## (Intercept)                      2.7172     0.2465 22.8582
## PopulationPortugal                1.1157     0.3324 44.6909
## Heat_TreatmentMHW2               -0.5689     0.3486 22.8582
## Heat_TreatmentMHW4                1.6060     0.3486 22.8582
## PhaseRecovery                    -0.2246     0.2806 27.0000
## PopulationPortugal:Heat_TreatmentMHW2  0.8047     0.4700 44.6909
## PopulationPortugal:Heat_TreatmentMHW4 -0.5393     0.4700 44.6909
## PopulationPortugal:PhaseRecovery      0.3564     0.3800 27.0000
## Heat_TreatmentMHW2:PhaseRecovery     -0.7286     0.3969 27.0000
## Heat_TreatmentMHW4:PhaseRecovery     -1.8594     0.3969 27.0000
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery  0.3581     0.5373 27.0000
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery  0.2637     0.5373 27.0000
##
##                                     t value Pr(>|t|)
## (Intercept)                      11.023 1.27e-10 ***
## PopulationPortugal                3.357 0.001618 **
## Heat_TreatmentMHW2               -1.632 0.116391
## Heat_TreatmentMHW4                4.607 0.000126 ***
## PhaseRecovery                    -0.800 0.430566
## PopulationPortugal:Heat_TreatmentMHW2  1.712 0.093847 .
## PopulationPortugal:Heat_TreatmentMHW4 -1.147 0.257328
## PopulationPortugal:PhaseRecovery      0.938 0.356625
## Heat_TreatmentMHW2:PhaseRecovery     -1.836 0.077402 .
## Heat_TreatmentMHW4:PhaseRecovery     -4.685 7.10e-05 ***
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery  0.666 0.510789
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery  0.491 0.627540
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PopltnPrtgl -0.739
## Ht_TrtnMHW2 -0.707  0.522
## Ht_TrtnMHW4 -0.707  0.522  0.500
## PhaseRecvry -0.574  0.424  0.406  0.406
## PpP:H_TMHW2  0.522 -0.707 -0.739 -0.369 -0.300
## PpP:H_TMHW4  0.522 -0.707 -0.369 -0.739 -0.300  0.500
## PpltnPrt:PR  0.424 -0.574 -0.300 -0.300 -0.739  0.406  0.406
## Ht_TMHW2:PR  0.406 -0.300 -0.574 -0.287 -0.707  0.424  0.212
## Ht_TMHW4:PR  0.406 -0.300 -0.287 -0.574 -0.707  0.212  0.424
## PP:H_TMHW2: -0.300  0.406  0.424  0.212  0.522 -0.574 -0.287
## PP:H_TMHW4: -0.300  0.406  0.212  0.424  0.522 -0.287 -0.574
##
##      PpP:PR H_TMHW2: H_TMHW4: PP:H_TMHW2:
## PopltnPrtgl
## Ht_TrtnMHW2
## Ht_TrtnMHW4
## PhaseRecvry
## PpP:H_TMHW2

```

```
## PpP:H_TMHw4
## PpltnPrt:PR
## Ht_TMHw2:PR 0.522
## Ht_TMHw4:PR 0.522 0.500
## PP:H_TMHw2: -0.707 -0.739 -0.369
## PP:H_TMHw4: -0.707 -0.369 -0.739 0.500
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
anova(lmer_SRec_Resp, type = 3, ddf="Kenward-Roger") #SRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##
## Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Population 17.5343 17.5343 1 24.4719 89.0661 1.249e-09
## Heat_Treatment 4.4238 2.2119 2 2.8822 11.2354 0.043567
## Phase 10.6145 10.6145 1 27.0000 53.9168 6.731e-08
## Population:Heat_Treatment 2.7078 1.3539 2 24.4719 6.8772 0.004265
## Population:Phase 1.2996 1.2996 1 27.0000 6.6014 0.016032
## Heat_Treatment:Phase 8.4978 4.2489 2 27.0000 21.5824 2.516e-06
## Population:Heat_Treatment:Phase 0.0940 0.0470 2 27.0000 0.2386 0.789358
##
## Population ***
## Heat_Treatment *
## Phase ***
## Population:Heat_Treatment **
## Population:Phase *
## Heat_Treatment:Phase ***
## Population:Heat_Treatment:Phase
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.3.4. Post-HOC analysis

For the Large MHW:

1. Simple two-way interaction analysis: There was a statistically significant simple two-way interaction between MHW intensity and population for the MHW end ($F[2, 24] = 40.20$, $p < 0.001$).
2. Simple-simple main effect analysis: There was a statistically significant simple simple main effect of the MHW intensity on the Respiration rate for the Cantabrian ($F[2, 3] = 31.2$, $p = 0.013$).
3. Simple comparisons: Atlantic population experienced a non-significant increase on the respiration rate after the MHW which turned significant after the recovery.

```
two.way_LRec1_Resp <- twoway2(Data = Resp_Stats_LMHW_NO, subset = "MHW")
two.way_LRec2_Resp <- twoway2(Data = Resp_Stats_LMHW_NO, subset = "Recovery")
bind_rows(two.way_LRec1_Resp, two.way_LRec2_Resp) #LRec
```

```
## # A tibble: 6 x 9
## Phase term sumsq meansq NumDF DenDF statistic p.value Signif
## <chr> <chr> <dbl> <dbl> <int> <dbl> <dbl> <dbl> <chr>
## 1 MHW Heat_Treatment 0.0288 0.0144 2 2.88 3.67 1.61e-1 ""
```

```
## 2 MHW      Population      0.343 0.343      1 24.5      87.3 1.52e-9 "*"
## 3 MHW      Heat_Treatment:Po~ 0.316 0.158      2 24.5      40.2 1.85e-8 "*"
## 4 Recovery Heat_Treatment      0.0601 0.0301      2 2.88      3.08 1.92e-1 ""
## 5 Recovery Population      0.0913 0.0913      1 24.5      9.35 5.32e-3 "*"
## 6 Recovery Heat_Treatment:Po~ 0.0431 0.0216      2 24.5      2.21 1.31e-1 ""
```

```
simple_LRec1_Resp <- simpleeffects2(Data = Resp_Stats_LMHW_NO,
                                   subset = c("MHW", "Cantabria"))
simple_LRec2_Resp <- simpleeffects2(Data = Resp_Stats_LMHW_NO,
                                   subset = c("Recovery", "Cantabria"))
simple_LRec3_Resp <- simpleeffects2(Data = Resp_Stats_LMHW_NO,
                                   subset = c("MHW", "Portugal"))
simple_LRec4_Resp <- simpleeffects2(Data = Resp_Stats_LMHW_NO,
                                   subset = c("Recovery", "Portugal"))
simple_LRec_Resp <- bind_rows(simple_LRec1_Resp, simple_LRec2_Resp,
                             simple_LRec3_Resp, simple_LRec4_Resp)

pwc_LRec_Resp <- emmeans(lmer_LRec_Resp, ~ Heat_Treatment | Phase * Population) %>%
  pairs(adjust="Tukey")
pwc_LRec_Resp
```

```
## Phase = MHW, Population = Cantabria:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2 -0.09715 0.0503 21.1  -1.930 0.1550
## Control - MHW4  0.20994 0.0528 23.2   3.976 0.0016
## MHW2 - MHW4     0.30709 0.0503 21.1   6.100 <.0001
##
## Phase = Recovery, Population = Cantabria:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2 -0.03752 0.0503 21.1  -0.745 0.7396
## Control - MHW4 -0.03184 0.0528 23.2  -0.603 0.8199
## MHW2 - MHW4     0.00569 0.0503 21.1   0.113 0.9930
##
## Phase = MHW, Population = Portugal:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2  0.07926 0.0503 21.1   1.574 0.2782
## Control - MHW4 -0.09295 0.0478 18.9  -1.946 0.1534
## MHW2 - MHW4    -0.17221 0.0503 21.1  -3.421 0.0069
##
## Phase = Recovery, Population = Portugal:
## contrast      estimate      SE    df t.ratio p.value
## Control - MHW2 -0.00709 0.0503 21.1  -0.141 0.9891
## Control - MHW4 -0.16957 0.0478 18.9  -3.551 0.0058
## MHW2 - MHW4    -0.16248 0.0503 21.1  -3.228 0.0107
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

For the Short MHW:

1. Simple two-way interaction analysis: It was not computed considering that there was not significant three-way interactions among treatments.

2. Simple-simple main effect analysis: There was a statistically significant simple simple main effect of the MHW intensity on the Respiration rates for Cantabrian population after MHW and Recovery (MHW: $F[2, 3] = 14.6$, $p = 0.032$; Rec: $F[2, 3] = 15.5$, $p = 0.037$).
3. Simple comparisons: Respiration rates were significantly increased by the high intensity MHW for the Cantabrian and Atlantic populations after the MHW, but reached control levels after the Recovery period.

```

simple_SRec1_Resp <- simpleeffects(Data = Resp_Stats_SMHW_NO,
                                  subset = c("MHW", "Cantabria"))
simple_SRec2_Resp <- simpleeffects(Data = Resp_Stats_SMHW_NO,
                                  subset = c("Recovery", "Cantabria"))
simple_SRec3_Resp <- simpleeffects(Data = Resp_Stats_SMHW_NO,
                                  subset = c("MHW", "Portugal"))
simple_SRec4_Resp <- simpleeffects(Data = Resp_Stats_SMHW_NO,
                                  subset = c("Recovery", "Portugal"))
simple_SRec_Resp <- bind_rows(simple_SRec1_Resp, simple_SRec2_Resp,
                             simple_SRec3_Resp, simple_SRec4_Resp)
simple_SRec_Resp

## # A tibble: 4 x 10
##   Phase      Population term      sumsq meansq NumDF DenDF statistic p.value Signif
##   <chr>      <chr>      <chr>      <dbl>  <dbl>  <int>  <dbl>      <dbl>  <dbl> <chr>
## 1 MHW      Cantabria  Heat_Tr~  7.47   3.74     2    2.81      14.6   0.0325 "*"
## 2 Recovery Cantabria  Heat_Tr~  4.40   2.20     2    2.55      15.5   0.0373 "*"
## 3 MHW      Portugal   Heat_Tr~  3.77   1.88     2    3.00       4.93  0.113  ""
## 4 Recovery Portugal   Heat_Tr~  0.907  0.453     2    3.00       1.32  0.389  ""

pwc_SRec_Resp <- emmeans(lmer_SRec_Resp, ~ Heat_Treatment | Phase * Population) %>%
  pairs(adjust="Tukey")
pwc_SRec_Resp

## Phase = MHW, Population = Cantabria:
##   contrast      estimate      SE    df t.ratio p.value
## Control - MHW2    0.569 0.349 22.9   1.632 0.2529
## Control - MHW4   -1.606 0.349 22.9  -4.607 0.0004
## MHW2 - MHW4      -2.175 0.349 22.9  -6.239 <.0001
##
## Phase = Recovery, Population = Cantabria:
##   contrast      estimate      SE    df t.ratio p.value
## Control - MHW2    1.298 0.349 22.9   3.722 0.0031
## Control - MHW4    0.253 0.349 22.9   0.727 0.7504
## MHW2 - MHW4      -1.044 0.349 22.9  -2.995 0.0172
##
## Phase = MHW, Population = Portugal:
##   contrast      estimate      SE    df t.ratio p.value
## Control - MHW2   -0.236 0.315 18.6  -0.748 0.7387
## Control - MHW4   -1.067 0.315 18.6  -3.383 0.0086
## MHW2 - MHW4      -0.831 0.315 18.6  -2.635 0.0417
##
## Phase = Recovery, Population = Portugal:
##   contrast      estimate      SE    df t.ratio p.value
## Control - MHW2    0.135 0.315 18.6   0.427 0.9048

```

```
## Control - MHW4      0.529 0.315 18.6   1.677 0.2400
## MHW2 - MHW4        0.394 0.315 18.6   1.250 0.4398
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

2.4. Compensation point analysis

2.4.1. Prepare data

```
Ic_Stats <- PI[PI$Parameter == "Ic",]
Ic_Stats$ID <- as.factor(Ic_Stats$ID)
Ic_Stats$Phase <- as.factor(Ic_Stats$Phase)
Ic_Stats$Tank <- as.factor(Ic_Stats$Tank)
Ic_Stats$Heat_Treatment <- as.factor(Ic_Stats$Heat_Treatment)
Ic_Stats$Population <- as.factor(Ic_Stats$Population)
Ic_Stats$Value_Log <- log10(Ic_Stats$Value + 1)
summary(Ic_Stats)
```

```
##      Day      Phase  Duration_Treatment  Population
## Length:139      MHW      :69  Length:139      Cantabria:72
## Class :character  Recovery:70  Class :character  Portugal :67
## Mode  :character      Mode  :character
##
##
##
## Heat_Treatment  Sensor      ID      Parameter
## Control:48      Length:139      1      : 4  Length:139
## MHW2  :48      Class :character  2      : 4  Class :character
## MHW4  :43      Mode  :character  3      : 4  Mode  :character
##
##      4      : 4
##      5      : 4
##      6      : 4
##      (Other):115
##      Value      Tank      Value_Log
## Min.   : 7.198  1      :12  Min.   :0.9137
## 1st Qu.: 34.603  2      :12  1st Qu.:1.5513
## Median : 50.022  3      :12  Median :1.7078
## Mean   : 55.930  4      :12  Mean   :1.6885
## 3rd Qu.: 77.193  5      :12  3rd Qu.:1.8932
## Max.   :141.988  7      :12  Max.   :2.1553
##      (Other):67
```

```
Ic_Stats_SMHW <- Ic_Stats[Ic_Stats$Duration_Treatment == "Srec",]
Ic_Stats_LMHW <- Ic_Stats[Ic_Stats$Duration_Treatment == "Lrec",]
```

2.4.2. Check Model assumptions

2.4.2.1. Outliers

First of all, the outliers were identified and removed when considered extreme.

```

# Large MHW
outliers_LMHW <- Ic_Stats_LMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Value)
outliers_LMHW <- outliers_LMHW[-c(11,12)]
Ic_Stats_LMHW_NO <- anti_join(Ic_Stats_LMHW, outliers_LMHW, by = c("ID"))

# Short MHW
outliers_SMHW <- Ic_Stats_SMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Value)
outliers_SMHW <- outliers_SMHW[-c(11,12)]
Ic_Stats_SMHW_NO <- anti_join(Ic_Stats_SMHW, outliers_SMHW, by = c("ID"))

```

2.4.2.2. Normality

The second step was to test normality. For that purpose, we computed Shapiro-Wilk's tests for each combinations of factor levels. Normality was also assessed visually by inspecting qqplots. According to the results, no clear deviations from normality were observed.

```

#Large MHW
Ic_Stats_LMHW_NO %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  shapiro_test(Value)

```

```

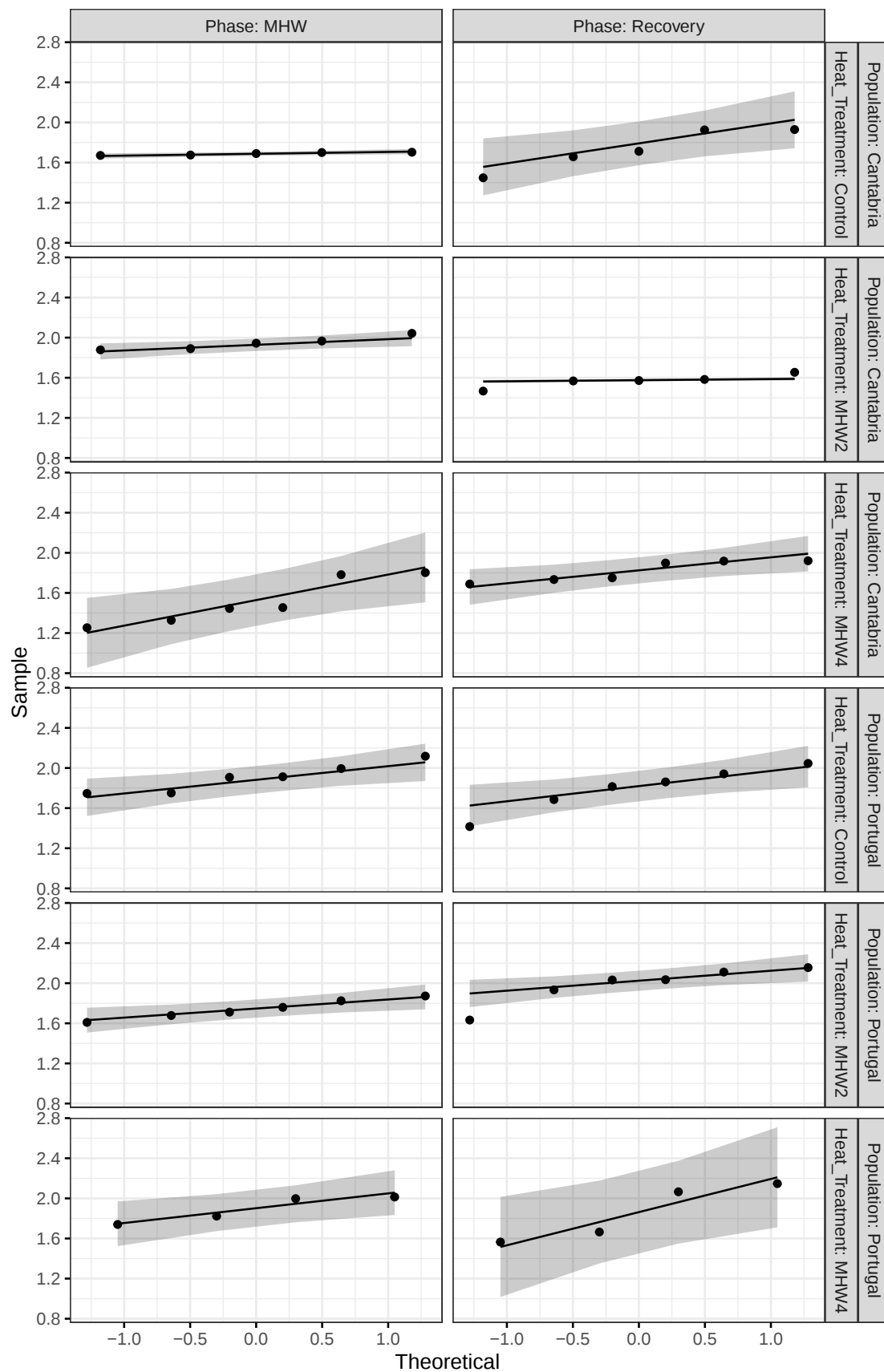
## # A tibble: 12 x 6
##   Phase      Population Heat_Treatment variable statistic      p
##   <fct>      <fct>      <fct>      <chr>      <dbl> <dbl>
## 1 MHW        Cantabria   Control    Value        0.898 0.396
## 2 Recovery  Cantabria   Control    Value        0.888 0.345
## 3 MHW        Cantabria   MHW2       Value        0.917 0.511
## 4 Recovery  Cantabria   MHW2       Value        0.929 0.591
## 5 MHW        Cantabria   MHW4       Value        0.806 0.0661
## 6 Recovery  Cantabria   MHW4       Value        0.818 0.0846
## 7 MHW        Portugal    Control    Value        0.909 0.433
## 8 Recovery  Portugal    Control    Value        0.997 1.00
## 9 MHW        Portugal    MHW2       Value        0.974 0.919
## 10 Recovery Portugal    MHW2       Value        0.940 0.661
## 11 MHW        Portugal    MHW4       Value        0.868 0.290
## 12 Recovery Portugal    MHW4       Value        0.872 0.305

```

```

ggqqplot(Ic_Stats_LMHW_NO, "Value_Log", ggtheme = theme_bw()) +
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")

```



```
#Short MHW
```

```
Ic_Stats_SMHW_NO %>%
```

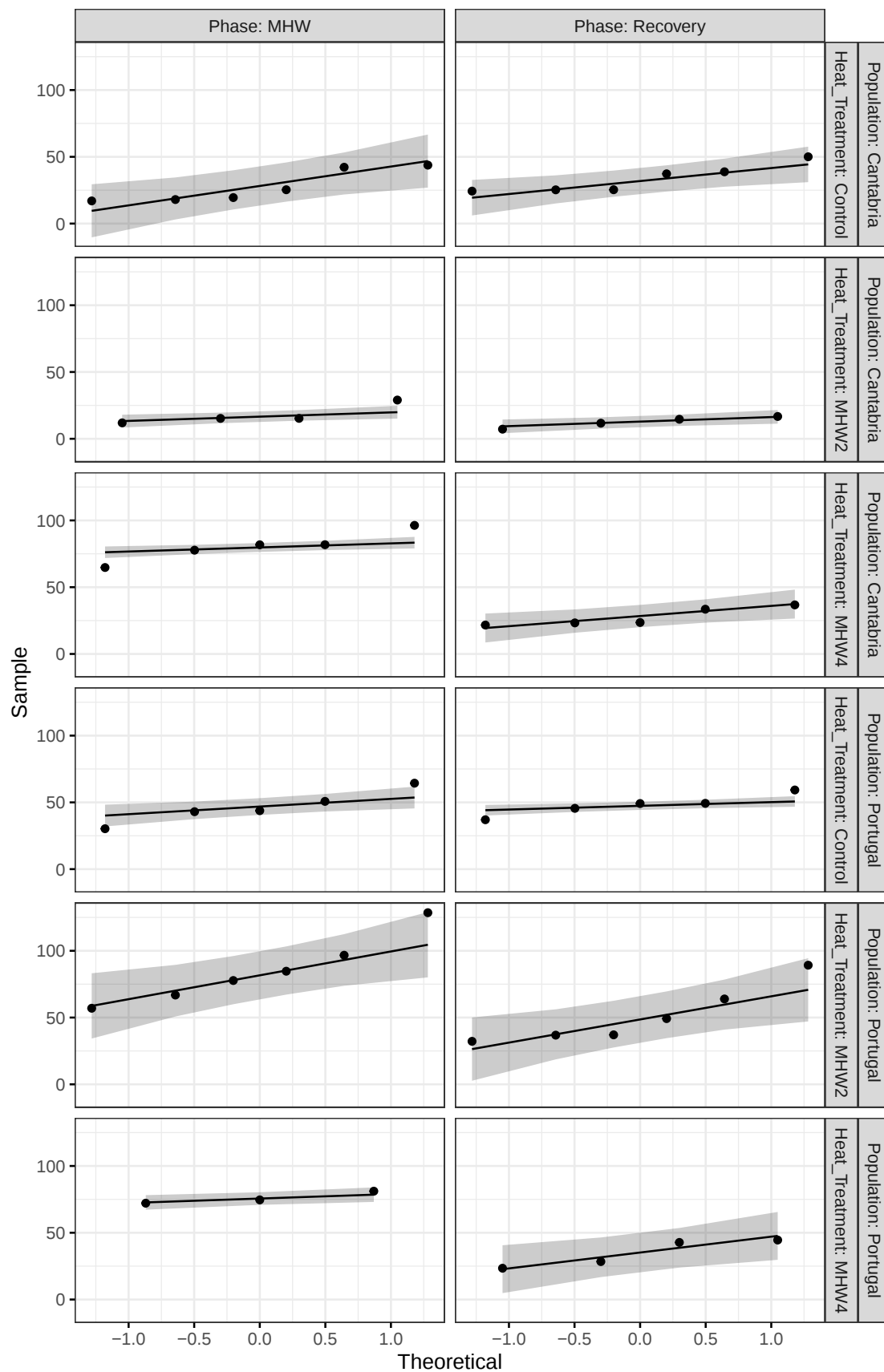
```
  group_by(Population, Heat_Treatment, Phase) %>%
```

```
  shapiro_test(Value)
```

```
## # A tibble: 12 x 6
```

##	Phase	Population	Heat_Treatment	variable	statistic	p
##	<fct>	<fct>	<fct>	<chr>	<dbl>	<dbl>
## 1	MHW	Cantabria	Control	Value	0.802	0.0613
## 2	Recovery	Cantabria	Control	Value	0.856	0.177
## 3	MHW	Cantabria	MHW2	Value	0.798	0.0991
## 4	Recovery	Cantabria	MHW2	Value	0.968	0.830
## 5	MHW	Cantabria	MHW4	Value	0.948	0.721
## 6	Recovery	Cantabria	MHW4	Value	0.828	0.133
## 7	MHW	Portugal	Control	Value	0.970	0.876
## 8	Recovery	Portugal	Control	Value	0.960	0.806
## 9	MHW	Portugal	MHW2	Value	0.946	0.705
## 10	Recovery	Portugal	MHW2	Value	0.864	0.204
## 11	MHW	Portugal	MHW4	Value	0.936	0.513
## 12	Recovery	Portugal	MHW4	Value	0.870	0.296

```
ggqqplot(Ic_Stats_SMHW_NO, "Value", ggtheme = theme_bw()) +  
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")
```



2.4.2.3. Homogeneity of variances

Homogeneity of variances was checked through Levene's tests. Tests using the complete dataset did not reveal clear heteroscedasticity for any MHW duration (Large/Short).

```
#Large MHW
```

```
Ic_Stats_LMHW_NO %>%  
  levene_test(Value_Log ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4  
##   df1 df2 statistic      p  
##   <int> <int>      <dbl> <dbl>  
## 1    11    52      2.16 0.0318
```

```
Ic_Stats_LMHW_NO %>%  
  group_by(Phase) %>%  
  levene_test(Value_Log ~ Heat_Treatment*Population)
```

```
## # A tibble: 2 x 5  
##   Phase      df1 df2 statistic      p  
##   <fct>   <int> <int>      <dbl> <dbl>  
## 1 MHW          5    26      2.36 0.0686  
## 2 Recovery     5    26      1.92 0.126
```

```
Ic_Stats_LMHW_NO %>%  
  group_by(Phase, Population) %>%  
  levene_test(Value_Log ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6  
##   Phase      Population df1 df2 statistic      p  
##   <fct>   <fct>      <int> <int>      <dbl> <dbl>  
## 1 MHW      Cantabria     2    13      3.99 0.0446  
## 2 MHW      Portugal      2    13      0.433 0.658  
## 3 Recovery Cantabria     2    13      2.98 0.0860  
## 4 Recovery Portugal      2    13      1.14 0.348
```

```
#Short MHW
```

```
Ic_Stats_SMHW_NO %>%  
  levene_test(Value ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4  
##   df1 df2 statistic      p  
##   <int> <int>      <dbl> <dbl>  
## 1    11    47      1.34 0.231
```

```
Ic_Stats_SMHW_NO %>%  
  group_by(Phase) %>%  
  levene_test(Value ~ Heat_Treatment*Population)
```

```
## # A tibble: 2 x 5  
##   Phase      df1 df2 statistic      p  
##   <fct>   <int> <int>      <dbl> <dbl>  
## 1 MHW          5    23      1.34 0.283  
## 2 Recovery     5    24      1.63 0.190
```

```
Ic_Stats_SMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(Value ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Phase      Population    df1    df2 statistic      p
##   <fct>      <fct>      <int> <int>    <dbl> <dbl>
## 1 MHW        Cantabria         2     12     0.510 0.613
## 2 MHW        Portugal          2     11     1.73  0.223
## 3 Recovery Cantabria         2     12     1.58  0.246
## 4 Recovery Portugal          2     12     1.45  0.273
```

2.4.3. Mixed model

For the long MHW, there was a significant ($p < .05$) three-way interaction between time, population, and MHW intensity ($F[2, 27] = 17.911$, $p < 0.001$). From the model for the Short MHW what we observed was that there was not a statistically significant ($p > .05$) three-way interaction between time, population, and MHW intensity ($F[2, 24] = 2.388$, $p = 0.113$), but there was a significant two-way interactions between Time/MHW intensity ($F[2, 24] = 15.746$, $p < 0.001$) and Population/MHW intensity ($F[2, 22] = 16.127$, $p < 0.001$).

```
#LRec
lmer_LRec_Ic <- lmerTest::lmer(Value_Log ~ Population*Heat_Treatment*Phase
  + (1 | Tank) + (1 | ID),
  Ic_Stats_LMHW_NO, REML = TRUE)
summary(lmer_LRec_Ic, ddf="Kenward-Roger") #SRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: Value_Log ~ Population * Heat_Treatment * Phase + (1 | Tank) +
##          (1 | ID)
## Data: Ic_Stats_LMHW_NO
##
## REML criterion at convergence: -26.9
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.40956 -0.50658  0.07565  0.56706  1.53102
##
## Random effects:
##  Groups   Name                Variance Std.Dev.
##  ID       (Intercept)  0.001683  0.04102
##  Tank     (Intercept)  0.009058  0.09517
##  Residual                    0.020051  0.14160
## Number of obs: 64, groups: ID, 33; Tank, 6
##
## Fixed effects:
##                                     Estimate Std. Error
## (Intercept)                        1.68407    0.09446
## PopulationPortugal                  0.22093    0.08954
## Heat_TreatmentMHW2                  0.26655    0.13359
## Heat_TreatmentMHW4                 -0.17349    0.13067
```

```

## PhaseRecovery 0.04656 0.08956
## PopulationPortugal:Heat_TreatmentMHW2 -0.42914 0.12662
## PopulationPortugal:Heat_TreatmentMHW4 0.21445 0.13218
## PopulationPortugal:PhaseRecovery -0.15757 0.12126
## Heat_TreatmentMHW2:PhaseRecovery -0.42188 0.12665
## Heat_TreatmentMHW4:PhaseRecovery 0.26092 0.12126
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 0.77352 0.17149
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery -0.23217 0.17917
## df t value Pr(>|t|)
## (Intercept) 7.26843 17.828 2.9e-07
## PopulationPortugal 48.16661 2.467 0.017212
## Heat_TreatmentMHW2 7.26843 1.995 0.084708
## Heat_TreatmentMHW4 6.69973 -1.328 0.227725
## PhaseRecovery 25.40483 0.520 0.607611
## PopulationPortugal:Heat_TreatmentMHW2 48.16661 -3.389 0.001407
## PopulationPortugal:Heat_TreatmentMHW4 48.64452 1.622 0.111185
## PopulationPortugal:PhaseRecovery 25.40483 -1.299 0.205451
## Heat_TreatmentMHW2:PhaseRecovery 25.40483 -3.331 0.002652
## Heat_TreatmentMHW4:PhaseRecovery 25.40483 2.152 0.041111
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 25.40483 4.511 0.000128
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 27.65064 -1.296 0.205753
##
## (Intercept) ***
## PopulationPortugal *
## Heat_TreatmentMHW2 .
## Heat_TreatmentMHW4
## PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW2 **
## PopulationPortugal:Heat_TreatmentMHW4
## PopulationPortugal:PhaseRecovery
## Heat_TreatmentMHW2:PhaseRecovery **
## Heat_TreatmentMHW4:PhaseRecovery *
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery ***
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
## (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PopltnPrtgl -0.519
## Ht_TrtnMHW2 -0.707 0.367
## Ht_TrtnMHW4 -0.723 0.375 0.511
## PhaseRecvry -0.474 0.501 0.335 0.343
## PpP:H_TMHW2 0.367 -0.707 -0.519 -0.265 -0.354
## PpP:H_TMHW4 0.353 -0.681 -0.250 -0.466 -0.341 0.481
## PpltnPrt:PR 0.350 -0.678 -0.248 -0.253 -0.739 0.479 0.461
## Ht_TMHW2:PR 0.335 -0.354 -0.474 -0.242 -0.707 0.501 0.241
## Ht_TMHW4:PR 0.350 -0.370 -0.248 -0.464 -0.739 0.261 0.461
## PP:H_TMHW2: -0.248 0.479 0.350 0.179 0.522 -0.678 -0.326
## PP:H_TMHW4: -0.238 0.461 0.168 0.316 0.502 -0.326 -0.683
## PpP:PR H_TMHW2: H_TMHW4: PP:H_TMHW2:
## PopltnPrtgl
## Ht_TrtnMHW2
## Ht_TrtnMHW4

```

```
## PhaseRecvry
## PpP:H_TMHw2
## PpP:H_TMHw4
## PpltnPrt:PR
## Ht_TMHw2:PR 0.522
## Ht_TMHw4:PR 0.545 0.522
## PP:H_TMHw2: -0.707 -0.739 -0.386
## PP:H_TMHw4: -0.680 -0.355 -0.680 0.481
```

```
anova(lmer_LRec_Ic, type = 3, ddf="Kenward-Roger") #LRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
##
##              Sum Sq Mean Sq NumDF   DenDF F value    Pr(>F)
## Population          0.34243  0.34243     1 24.0611 17.0783 0.0003751
## Heat_Treatment        0.00240  0.00120     2  2.9956  0.0599 0.9429689
## Phase                0.00029  0.00029     1 26.9214  0.0145 0.9049843
## Population:Heat_Treatment 0.04443  0.02221     2 23.9894  1.1078 0.3466014
## Population:Phase        0.00202  0.00202     1 26.9214  0.1006 0.7535349
## Heat_Treatment:Phase    0.08882  0.04441     2 26.8209  2.2148 0.1287559
## Population:Heat_Treatment:Phase 0.71829  0.35915     2 26.8209 17.9108 1.147e-05
##
## Population          ***
## Heat_Treatment
## Phase
## Population:Heat_Treatment
## Population:Phase
## Heat_Treatment:Phase
## Population:Heat_Treatment:Phase ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#SRec
lmer_SRec_Ic <- lmerTest::lmer(Value ~ Population*Heat_Treatment*Phase
                               + (1 | Tank) + (1 | ID),
                               Ic_Stats_SMHW_NO, REML = TRUE)
summary(lmer_LRec_Ic, ddf="Kenward-Roger") #SRec
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger's method [
## lmerModLmerTest]
## Formula: Value_Log ~ Population * Heat_Treatment * Phase + (1 | Tank) +
##          (1 | ID)
## Data: Ic_Stats_LMHW_NO
##
## REML criterion at convergence: -26.9
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.40956 -0.50658  0.07565  0.56706  1.53102
##
## Random effects:
## Groups   Name                Variance Std.Dev.
## ID      (Intercept) 0.001683 0.04102
## Tank    (Intercept) 0.009058 0.09517
```

```

## Residual          0.020051 0.14160
## Number of obs: 64, groups: ID, 33; Tank, 6
##
## Fixed effects:
##
##                                     Estimate Std. Error
## (Intercept)                        1.68407    0.09446
## PopulationPortugal                  0.22093    0.08954
## Heat_TreatmentMHW2                  0.26655    0.13359
## Heat_TreatmentMHW4                 -0.17349    0.13067
## PhaseRecovery                      0.04656    0.08956
## PopulationPortugal:Heat_TreatmentMHW2 -0.42914    0.12662
## PopulationPortugal:Heat_TreatmentMHW4  0.21445    0.13218
## PopulationPortugal:PhaseRecovery     -0.15757    0.12126
## Heat_TreatmentMHW2:PhaseRecovery     -0.42188    0.12665
## Heat_TreatmentMHW4:PhaseRecovery      0.26092    0.12126
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery  0.77352    0.17149
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery -0.23217    0.17917
##
##                                     df t value Pr(>|t|)
## (Intercept)                        7.26843  17.828  2.9e-07
## PopulationPortugal                48.16661   2.467  0.017212
## Heat_TreatmentMHW2                 7.26843   1.995  0.084708
## Heat_TreatmentMHW4                 6.69973  -1.328  0.227725
## PhaseRecovery                     25.40483   0.520  0.607611
## PopulationPortugal:Heat_TreatmentMHW2 48.16661  -3.389  0.001407
## PopulationPortugal:Heat_TreatmentMHW4 48.64452   1.622  0.111185
## PopulationPortugal:PhaseRecovery     25.40483  -1.299  0.205451
## Heat_TreatmentMHW2:PhaseRecovery     25.40483  -3.331  0.002652
## Heat_TreatmentMHW4:PhaseRecovery     25.40483   2.152  0.041111
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery 25.40483   4.511  0.000128
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery 27.65064  -1.296  0.205753
##
## (Intercept)                        ***
## PopulationPortugal                  *
## Heat_TreatmentMHW2                  .
## Heat_TreatmentMHW4
## PhaseRecovery
## PopulationPortugal:Heat_TreatmentMHW2      **
## PopulationPortugal:Heat_TreatmentMHW4
## PopulationPortugal:PhaseRecovery
## Heat_TreatmentMHW2:PhaseRecovery      **
## Heat_TreatmentMHW4:PhaseRecovery          *
## PopulationPortugal:Heat_TreatmentMHW2:PhaseRecovery ***
## PopulationPortugal:Heat_TreatmentMHW4:PhaseRecovery
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) PpltnP Ht_TMHW2 Ht_TMHW4 PhsRcv PpP:H_TMHW2 PpP:H_TMHW4
## PopltnPrtgl -0.519
## Ht_TrtmMHW2 -0.707  0.367
## Ht_TrtmMHW4 -0.723  0.375  0.511
## PhaseRecvry -0.474  0.501  0.335   0.343
## PpP:H_TMHW2  0.367 -0.707 -0.519  -0.265  -0.354
## PpP:H_TMHW4  0.353 -0.681 -0.250  -0.466  -0.341  0.481

```

```
## PpltnPrt:PR 0.350 -0.678 -0.248 -0.253 -0.739 0.479 0.461
## Ht_TmHW2:PR 0.335 -0.354 -0.474 -0.242 -0.707 0.501 0.241
## Ht_TmHW4:PR 0.350 -0.370 -0.248 -0.464 -0.739 0.261 0.461
## PP:H_TmHW2: -0.248 0.479 0.350 0.179 0.522 -0.678 -0.326
## PP:H_TmHW4: -0.238 0.461 0.168 0.316 0.502 -0.326 -0.683
## PpP:PR H_TmHW2: H_TmHW4: PP:H_TmHW2:
## PopltnPrtgl
## Ht_TrtmMHW2
## Ht_TrtmMHW4
## PhaseRecvry
## PpP:H_TmHW2
## PpP:H_TmHW4
## PpltnPrt:PR
## Ht_TmHW2:PR 0.522
## Ht_TmHW4:PR 0.545 0.522
## PP:H_TmHW2: -0.707 -0.739 -0.386
## PP:H_TmHW4: -0.680 -0.355 -0.680 0.481
```

```
anova(lmer_SRec_Ic, type = 3, ddf="Kenward-Roger") #SRec
```

```
## Type III Analysis of Variance Table with Kenward-Roger's method
## Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Population 7631.4 7631.4 1 21.9267 40.2364 2.239e-06
## Heat_Treatment 1902.7 951.3 2 2.6819 5.0000 0.1245
## Phase 6166.3 6166.3 1 24.0262 32.5117 7.094e-06
## Population:Heat_Treatment 6118.1 3059.0 2 21.8594 16.1272 4.981e-05
## Population:Phase 178.2 178.2 1 24.0262 0.9394 0.3421
## Heat_Treatment:Phase 5973.1 2986.6 2 23.9628 15.7463 4.304e-05
## Population:Heat_Treatment:Phase 905.7 452.8 2 23.9628 2.3875 0.1134
##
## Population ***
## Heat_Treatment
## Phase ***
## Population:Heat_Treatment ***
## Population:Phase
## Heat_Treatment:Phase ***
## Population:Heat_Treatment:Phase
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.4.4. Post-HOC analysis

For the Large MHW:

1. Simple two-way interaction analysis: There was a statistically significant simple two-way interaction between MHW intensity and population for the MHW end ($F[2, 23] = 22.0$, $p < 0.001$) and Recovery ($F[2, 23] = 3.79$, $p = 0.040$)
2. Simple-simple main effect analysis: No significant effects.
3. Simple comparisons: No significant differences.

```
two.way_LRec1_Ic <- twoway2(Data = Ic_Stats_LMHW_NO, subset = "MHW")
two.way_LRec2_Ic <- twoway2(Data = Ic_Stats_LMHW_NO, subset = "Recovery")
bind_rows(two.way_LRec1_Ic, two.way_LRec2_Ic) #LRec
```

```
## # A tibble: 6 x 9
##   Phase      term      sumsq meansq NumDF DenDF statistic p.value Signif
##   <chr>    <chr>    <dbl>  <dbl> <int> <dbl>    <dbl>  <dbl> <chr>
## 1 MHW      Heat_Treatment 0.0116 0.00580     2  2.99     0.476 6.62e-1 ""
## 2 MHW      Population    0.162 0.162     1 23.2     13.3 1.33e-3 "*"
## 3 MHW      Heat_Treatment:P~ 0.537 0.269     2 23.2     22.0 4.32e-6 "*"
## 4 Recovery Heat_Treatment 0.0127 0.00634     2  3.02     0.222 8.13e-1 ""
## 5 Recovery Population    0.236 0.236     1 23.1     8.26 8.54e-3 "*"
## 6 Recovery Heat_Treatment:P~ 0.217 0.109     2 23.1     3.79 3.76e-2 "*"

```

```
simple_LRec1_Ic <- simpleeffects2(Data = Ic_Stats_LMHW_NO,
                                subset = c("MHW", "Cantabria"))
simple_LRec2_Ic <- simpleeffects2(Data = Ic_Stats_LMHW_NO,
                                subset = c("Recovery", "Cantabria"))
simple_LRec3_Ic <- simpleeffects2(Data = Ic_Stats_LMHW_NO,
                                subset = c("MHW", "Portugal"))
simple_LRec4_Ic <- simpleeffects2(Data = Ic_Stats_LMHW_NO,
                                subset = c("Recovery", "Portugal"))
simple_LRec_Ic <- bind_rows(simple_LRec1_Ic, simple_LRec2_Ic,
                           simple_LRec3_Ic, simple_LRec4_Ic)
simple_LRec_Ic
```

```
## # A tibble: 4 x 10
##   Phase      Population term      sumsq meansq NumDF DenDF statistic p.value Signif
##   <chr>    <chr>    <chr>    <dbl>  <dbl> <int> <dbl>    <dbl>  <dbl> <chr>
## 1 MHW      Cantabria Heat_T~ 0.104 0.0521     2  2.99     4.72 0.119 ""
## 2 Recovery Cantabria Heat_T~ 0.0516 0.0258     2  2.97     2.09 0.271 ""
## 3 MHW      Portugal  Heat_T~ 0.0343 0.0172     2  2.85     1.54 0.351 ""
## 4 Recovery Portugal  Heat_T~ 0.0386 0.0193     2  3.02     0.497 0.651 ""

```

```
pwc_LRec_Ic <- emmeans(lmer_LRec_Ic, ~ Heat_Treatment | Phase * Population) %>%
  pairs(adjust="Tukey")
pwc_LRec_Ic
```

```
## Phase = MHW, Population = Cantabria:
## contrast      estimate      SE   df t.ratio p.value
## Control - MHW2 -0.2666 0.134 7.27 -1.995 0.1812
## Control - MHW4  0.1735 0.131 6.70  1.328 0.4272
## MHW2 - MHW4      0.4400 0.131 6.70  3.368 0.0300
##
## Phase = Recovery, Population = Cantabria:
## contrast      estimate      SE   df t.ratio p.value
## Control - MHW2  0.1553 0.134 7.27  1.163 0.5086
## Control - MHW4 -0.0874 0.131 6.70 -0.669 0.7883
## MHW2 - MHW4     -0.2428 0.131 6.70 -1.858 0.2232
##
## Phase = MHW, Population = Portugal:
```

```
## contrast      estimate    SE    df t.ratio p.value
## Control - MHW2    0.1626 0.128 6.15   1.273  0.4573
## Control - MHW4   -0.0410 0.136 7.61  -0.301  0.9516
## MHW2 - MHW4      -0.2035 0.136 7.61  -1.496  0.3449
##
## Phase = Recovery, Population = Portugal:
## contrast      estimate    SE    df t.ratio p.value
## Control - MHW2   -0.1890 0.128 6.15  -1.481  0.3622
## Control - MHW4   -0.0697 0.135 7.51  -0.517  0.8656
## MHW2 - MHW4       0.1193 0.135 7.51   0.885  0.6651
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

For the Short MHW:

1. Simple two-way interaction analysis: It was not computed considering that there was not significant three-way interactions among treatments.
2. Simple-simple main effect analysis: There was a statistically significant simple simple main effect of the MHW intensity on the Ic for Cantabrian population after MHW (MHW: $F[2, 3] = 27.7$, $p = 0.016$)
3. Simple comparisons: Ic values were significantly increased by the high intensity MHW for the Cantabrian and Atlantic populations after the MHW, and for the low intensity MHW for the Atlantic population. No significant differences after a recovery period.

```
simple_SRec1_Ic <- simpleeffects(Data = Ic_Stats_SMHW_NO,
                                subset = c("MHW", "Cantabria"))
simple_SRec2_Ic <- simpleeffects(Data = Ic_Stats_SMHW_NO,
                                subset = c("Recovery", "Cantabria"))
simple_SRec3_Ic <- simpleeffects(Data = Ic_Stats_SMHW_NO,
                                subset = c("MHW", "Portugal"))
simple_SRec4_Ic <- simpleeffects(Data = Ic_Stats_SMHW_NO,
                                subset = c("Recovery", "Portugal"))
simple_SRec_Ic <- bind_rows(simple_SRec1_Ic, simple_SRec2_Ic,
                           simple_SRec3_Ic, simple_SRec4_Ic)
simple_SRec_Ic
```

```
## # A tibble: 4 x 10
##   Phase    Population term      sumsq meansq NumDF DenDF statistic p.value Signif
##   <chr>    <chr>    <chr>    <dbl>  <dbl>  <int>  <dbl>    <dbl>  <dbl> <chr>
## 1 MHW      Cantabria Heat_Tr~ 5441.  2721.    2  2.70    27.7  0.0159 "*"
## 2 Recovery Cantabria Heat_Tr~  934.   467.    2  2.35     7.15 0.0999 ""
## 3 MHW      Portugal  Heat_Tr~ 4070.  2035.    2  2.90     5.64 0.100 ""
## 4 Recovery Portugal  Heat_Tr~  602.   301.    2  2.35     1.20 0.436 ""
```

```
pwc_SRec_Ic <- emmeans(lmer_SRec_Ic, ~ Heat_Treatment | Phase * Population) %>%
  pairs(adjust="Tukey")
pwc_SRec_Ic
```

```
## Phase = MHW, Population = Cantabria:
## contrast      estimate    SE    df t.ratio p.value
## Control - MHW2    9.93  9.33 21.6   1.064  0.5457
```

```

## Control - MHW4    -52.73  8.62 19.9  -6.114  <.0001
## MHW2 - MHW4       -62.65  9.69 23.3  -6.467  <.0001
##
## Phase = Recovery, Population = Cantabria:
## contrast          estimate    SE   df t.ratio p.value
## Control - MHW2     21.11  9.33 21.6   2.264  0.0830
## Control - MHW4      5.86  8.62 19.9   0.680  0.7779
## MHW2 - MHW4       -15.25  9.69 23.3  -1.574  0.2764
##
## Phase = MHW, Population = Portugal:
## contrast          estimate    SE   df t.ratio p.value
## Control - MHW2    -38.64  8.63 20.0  -4.478  0.0006
## Control - MHW4    -29.17 10.45 29.4  -2.792  0.0240
## MHW2 - MHW4        9.47 10.11 27.9   0.937  0.6219
##
## Phase = Recovery, Population = Portugal:
## contrast          estimate    SE   df t.ratio p.value
## Control - MHW2     -3.19  8.63 20.0  -0.370  0.9275
## Control - MHW4     13.70  9.68 23.2   1.416  0.3494
## MHW2 - MHW4        16.90  9.31 21.4   1.814  0.1888
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates

```

Tissue Damage Analysis

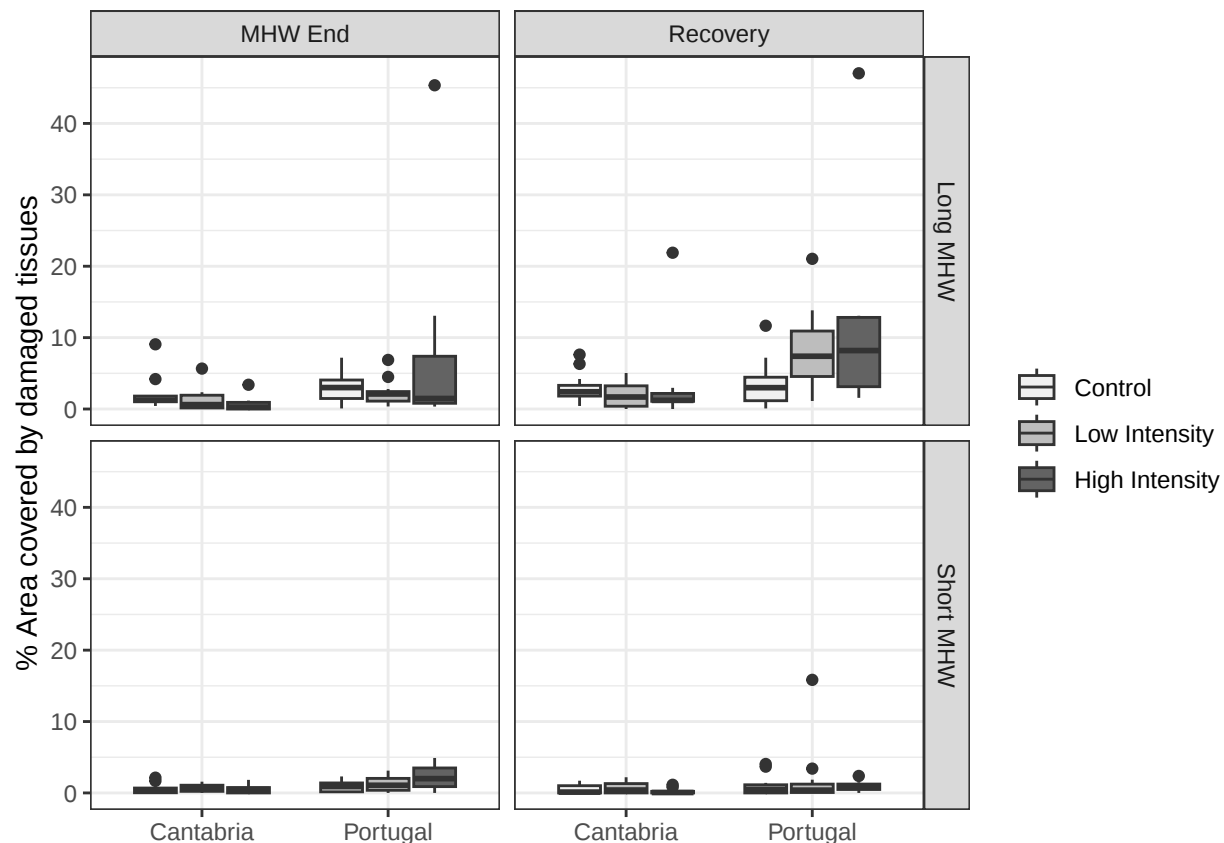
1. DATA PROCESSING

Data loading and inspection. First of all, we will visualize raw data using a boxplot.

```
# Data
Damage<-data.frame(read.xlsx("D:/Estancia_Porto_2023/experiments/MHW/Full_Datasets.xlsx",
                             sheet = 4))
Damage <- Damage[Damage$Duration_Treatment=="Srec"|Damage$Duration_Treatment == "Lrec",]
Damage <- Damage[,-c(10,11,12,14)]
Damage <- na.omit(Damage)

# Labels
day.labs <- c("MHW End", "Recovery")
names(day.labs) <- c("MHW", "Recovery")
Duration.labs <- c("Short MHW", "Long MHW")
names(Duration.labs) <- c("Srec", "Lrec")
MHW.labs <- c("Control", "Low Intensity", "High Intensity")

# Raw boxplot
Damage_plot <- ggplot(Damage, aes(x = as.factor(Population),
                                   y = Dam_Ref_1, fill = as.factor(Heat_Treatment))) +
  geom_boxplot()+
  facet_grid(Duration_Treatment~Phase,
             labeller = labeller(Duration_Treatment = Duration.labs, Phase = day.labs)) +
  labs(x = NULL, y = '% Area covered by damaged tissues', fill = NULL) +
  theme(text=element_text(family=windowsFont("Arial")),
        axis.text.x = element_text(color = "grey20", size = 14),
        axis.text.y = element_text(color = "grey20", size = 14),
        axis.title.x = element_text(color = "grey20", size = 16),
        axis.title.y = element_text(color = "grey20", size = 16),
        strip.text.x = element_text(color = "grey20", size = 16),
        strip.text.y = element_text(color = "grey20", size = 16),
        legend.text = element_text(color = "grey20", size = 14),
        legend.background = element_rect(colour = "grey20",
                                           fill = "white", size = 0.3)) +
  scale_fill_brewer(palette="Greys", labels = MHW.labs) + theme_bw()
Damage_plot
```



```
# Prepare data for statistical analysis
```

```
Damage_Stats <- Damage
Damage_Stats$ID <- as.factor(Damage_Stats$ID)
Damage_Stats$Tank <- as.factor(Damage_Stats$Tank)
Damage_Stats$Phase <- as.factor(Damage_Stats$Phase)
Damage_Stats$Heat_Treatment <- as.factor(Damage_Stats$Heat_Treatment)
Damage_Stats$Population <- as.factor(Damage_Stats$Population)
Damage_Stats$Dam_Ref_Log <- log10(Damage_Stats$Dam_Ref_1 + 1)
Damage_Stats_SMHW <- Damage_Stats[Damage_Stats$Duration_Treatment == "Srec",]
Damage_Stats_LMHW <- Damage_Stats[Damage_Stats$Duration_Treatment == "Lrec",]
summary(Damage_Stats_SMHW)
```

```
##      Day      Phase      Population Heat_Treatment Duration_Treatment
## Min.   : 7.00    MHW      :70    Cantabria:69    Control:46      Length:138
## 1st Qu.: 7.00    Recovery:68    Portugal :69    MHW2  :46      Class :character
## Median : 7.00                                MHW4   :46      Mode  :character
## Mean    :10.94
## 3rd Qu.:15.00
## Max.    :15.00
##
## Replicate      Tank      Plant      ID
## Length:138      4       :24    Min.   :1.000    1       : 2
## Class :character 6       :24    1st Qu.:2.000    2       : 2
## Mode  :character 1       :23    Median :3.000    3       : 2
##                2       :23    Mean   :3.471    4       : 2
##                3       :22    3rd Qu.:5.000    6       : 2
```

```
##           5      :22   Max.    :6.000   7      : 2
##           (Other): 0                (Other):126
##   Dam_Ref_1      Dam_Ref_Log
##   Min.    : 0.0000   Min.    :0.0000
##   1st Qu.: 0.0000   1st Qu.:0.0000
##   Median : 0.5298   Median :0.1846
##   Mean    : 0.9745   Mean    :0.2248
##   3rd Qu.: 1.3489   3rd Qu.:0.3709
##   Max.    :15.8392   Max.    :1.2263
##
```

```
summary(Damage_Stats_LMHW)
```

```
##           Day           Phase      Population Heat_Treatment Duration_Treatment
##   Min.    :21.00   MHW      :70   Cantabria:70   Control:48   Length:138
##   1st Qu.:21.00   Recovery:68   Portugal :68   MHW2    :47   Class :character
##   Median :21.00                MHW4    :43   Mode  :character
##   Mean    :24.45
##   3rd Qu.:28.00
##   Max.    :28.00
##
##   Replicate      Tank      Plant      ID
##   Length:138      7      :24   Min.    :1.000   1      : 2
##   Class :character 8      :24   1st Qu.:2.000   3      : 2
##   Mode  :character 10     :24   Median :4.000   4      : 2
##                9      :23   Mean    :3.507   5      : 2
##                12     :22   3rd Qu.:5.000   6      : 2
##                11     :21   Max.    :6.000   7      : 2
##                (Other): 0                (Other):126
##   Dam_Ref_1      Dam_Ref_Log
##   Min.    : 0.0000   Min.    :0.0000
##   1st Qu.: 0.7754   1st Qu.:0.2493
##   Median : 1.9319   Median :0.4671
##   Mean    : 3.8612   Mean    :0.5094
##   3rd Qu.: 4.0972   3rd Qu.:0.7071
##   Max.    :47.0259   Max.    :1.6815
##
```

2. STATISTICAL ANALYSIS

To test the effects of the different treatments (Long/Short, Low/High Intensity MHW) on the area covered by damaged tissues we will run a linear mixed-effects model. The analysis was performed separately for each MHW (Long/Short) as the results were compared to independent control treatments. In this context, **POPULATION** (w/ two levels: Atlantic and Cantabrian) and **MHW INTENSITY** (w/ three levels: Control, Low Intensity and High intensity) were considered between-subject factors, while **TIME** (w/ two levels: after MHW and after recovery period) was considered a within-subject factor. The response variable measured was the **Percentage area covered by damaged tissues from the total surface**. The aim was to understand how that area changes over time (After Stress Period vs Recovery) depending on the population of origin and the induced thermal stress imposed by the MHW (Low vs High vs Non-Stress).

2.1. Check Model assumptions

2.1.1. Outliers

First of all, the outliers were identified and removed when considered extreme.

```
# Large MHW
outliers_LMHW <- Damage_Stats_LMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Dam_Ref_1)
outliers_LMHW <- outliers_LMHW[-c(12,13)]
Damage_Stats_LMHW_NO <- anti_join(Damage_Stats_LMHW, outliers_LMHW, by = c("ID"))
outliers_LMHW %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, Dam_Ref_1) %>%
  head() # Large MHW outliers
```

```
## # A tibble: 6 x 7
##   Phase      Population Heat_Treatment    Day Duration_Treatment ID      Dam_Ref_1
##   <fct>      <fct>      <fct>      <dbl> <chr>              <fct>      <dbl>
## 1 MHW        Cantabria   Control        21 Lrec             50         9.07
## 2 MHW        Cantabria   Control        21 Lrec             53         4.19
## 3 Recovery  Cantabria   Control        28 Lrec             49         6.32
## 4 Recovery  Cantabria   Control        28 Lrec             50         7.62
## 5 MHW        Cantabria   MHW2           21 Lrec             25         5.66
## 6 MHW        Cantabria   MHW4           21 Lrec             10         3.40
```

```
# Short MHW
outliers_SMHW <- Damage_Stats_SMHW %>%
  group_by(Population, Heat_Treatment, Phase) %>%
  identify_outliers(Dam_Ref_1)
outliers_SMHW <- outliers_SMHW[-c(12,13)]
Damage_Stats_SMHW_NO <- anti_join(Damage_Stats_SMHW, outliers_SMHW, by = c("ID"))
outliers_SMHW %>%
  select(Phase, Population, Heat_Treatment, Day, Duration_Treatment, ID, Dam_Ref_1) %>%
  head() # Short MHW outliers
```

```
## # A tibble: 6 x 7
##   Phase      Population Heat_Treatment    Day Duration_Treatment ID      Dam_Ref_1
##   <fct>      <fct>      <fct>      <dbl> <chr>              <fct>      <dbl>
## 1 MHW        Cantabria   Control         7 Srec             49         1.69
## 2 MHW        Cantabria   Control         7 Srec             57         2.14
## 3 Recovery  Cantabria   MHW4           15 Srec             3          0.825
## 4 Recovery  Cantabria   MHW4           15 Srec             6          1.14
## 5 Recovery  Portugal    Control        15 Srec             66         3.68
## 6 Recovery  Portugal    Control        15 Srec             68         4.05
```

2.1.2. Normality

The second step was to test normality. For that purpose, we computed Shapiro-Wilk's tests for each combinations of factor levels. Normality was also assessed visually by inspecting qqplots. Shapiro-Wilk's tests and qqplots revealed normality issues for some combination.

```
# Large MMHW
```

```
Damage_Stats_LMHW_NO %>%
```

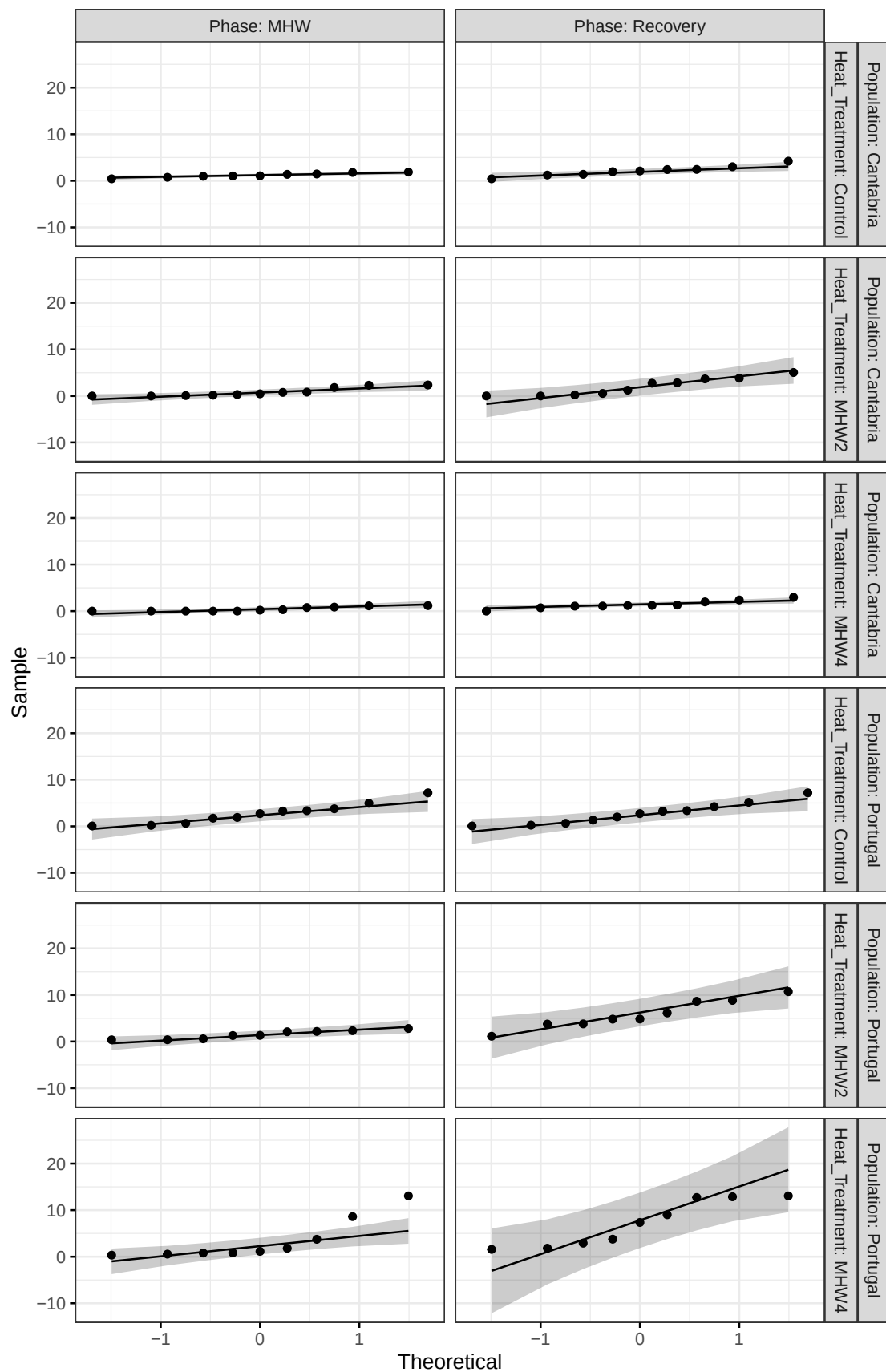
```
  group_by(Population, Heat_Treatment, Phase) %>%
```

```
  shapiro_test(Dam_Ref_1)
```

```
## # A tibble: 12 x 6
```

##	Phase	Population	Heat_Treatment	variable	statistic	p
##	<fct>	<fct>	<fct>	<chr>	<dbl>	<dbl>
##	1 MHW	Cantabria	Control	Dam_Ref_1	0.966	0.856
##	2 Recovery	Cantabria	Control	Dam_Ref_1	0.976	0.941
##	3 MHW	Cantabria	MHW2	Dam_Ref_1	0.820	0.0173
##	4 Recovery	Cantabria	MHW2	Dam_Ref_1	0.899	0.213
##	5 MHW	Cantabria	MHW4	Dam_Ref_1	0.792	0.00733
##	6 Recovery	Cantabria	MHW4	Dam_Ref_1	0.944	0.600
##	7 MHW	Portugal	Control	Dam_Ref_1	0.944	0.567
##	8 Recovery	Portugal	Control	Dam_Ref_1	0.949	0.628
##	9 MHW	Portugal	MHW2	Dam_Ref_1	0.909	0.307
##	10 Recovery	Portugal	MHW2	Dam_Ref_1	0.955	0.741
##	11 MHW	Portugal	MHW4	Dam_Ref_1	0.730	0.00318
##	12 Recovery	Portugal	MHW4	Dam_Ref_1	0.856	0.0875

```
ggqqplot(Damage_Stats_LMHW_NO, "Dam_Ref_1", ggtheme = theme_bw()) +  
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")
```



```
# Short MMHW
```

```
Damage_Stats_SMHW_NO %>%
```

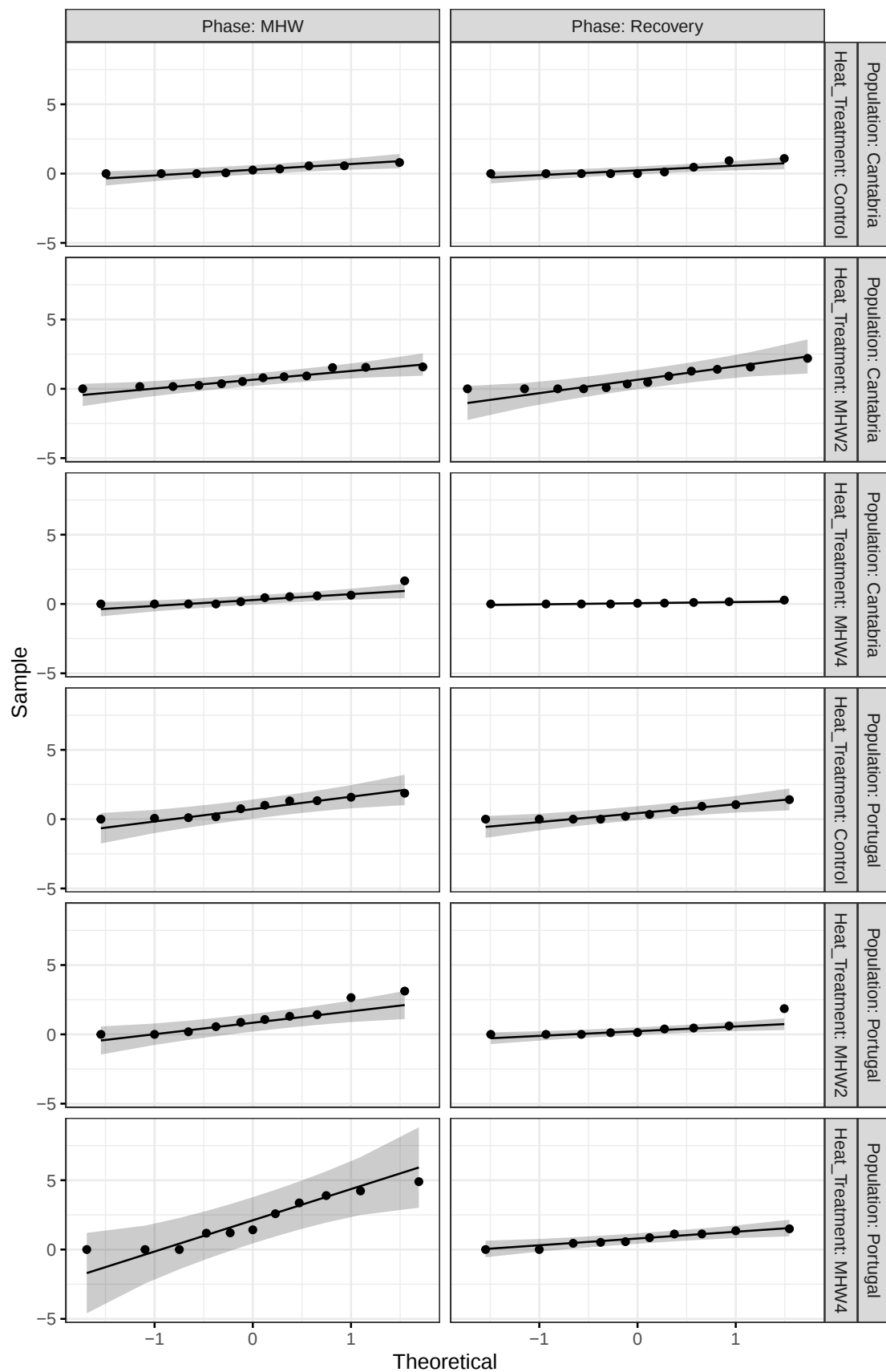
```
  group_by(Population, Heat_Treatment, Phase) %>%
```

```
  shapiro_test(Dam_Ref_1)
```

```
## # A tibble: 12 x 6
```

##	Phase	Population	Heat_Treatment	variable	statistic	p
##	<fct>	<fct>	<fct>	<chr>	<dbl>	<dbl>
## 1	MHW	Cantabria	Control	Dam_Ref_1	0.871	0.127
## 2	Recovery	Cantabria	Control	Dam_Ref_1	0.715	0.00213
## 3	MHW	Cantabria	MHW2	Dam_Ref_1	0.889	0.115
## 4	Recovery	Cantabria	MHW2	Dam_Ref_1	0.855	0.0421
## 5	MHW	Cantabria	MHW4	Dam_Ref_1	0.776	0.00730
## 6	Recovery	Cantabria	MHW4	Dam_Ref_1	0.810	0.0269
## 7	MHW	Portugal	Control	Dam_Ref_1	0.896	0.197
## 8	Recovery	Portugal	Control	Dam_Ref_1	0.852	0.0611
## 9	MHW	Portugal	MHW2	Dam_Ref_1	0.895	0.195
## 10	Recovery	Portugal	MHW2	Dam_Ref_1	0.707	0.00172
## 11	MHW	Portugal	MHW4	Dam_Ref_1	0.902	0.195
## 12	Recovery	Portugal	MHW4	Dam_Ref_1	0.936	0.514

```
ggqqplot(Damage_Stats_SMHW_NO, "Dam_Ref_1", ggtheme = theme_bw()) +  
  facet_grid(Population + Heat_Treatment ~ Phase, labeller = "label_both")
```



2.1.3. Homogeneity of variances

Homogeneity of variances was checked through Levene's tests. For the large MHW, tests using the complete dataset revealed clear heteroscedasticity. A deeper analysis, grouping data per Phase, showed that heterogeneity of variances was weak after the MHW ($0.05 > p > 0.01$) but strong for the Recovery ($p < 0.01$). For the short MHW, homogeneity was again not fully achieved. This heterogeneity was mainly motivated by the higher variances observed for Atlantic population after the MHW. In contrast to the large MHW, variations of the standard deviations were smaller. Finally, non-parametric transformations (Aligned Rank Transformation) were used to analyse data as normality and homogeneity of variances were not achieved.

```
# Large MMHW
Damage_Stats_LMHW_NO %>%
  levene_test(Dam_Ref_1 ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4
##   df1    df2 statistic      p
##   <int> <int>    <dbl>    <dbl>
## 1     11    106      5.02 0.00000292
```

```
Damage_Stats_LMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(Dam_Ref_1 ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Phase    Population    df1    df2 statistic      p
##   <fct>    <fct>      <int> <int>    <dbl>    <dbl>
## 1 MHW      Cantabria      2     28      1.40 0.263
## 2 MHW      Portugal       2     26      1.48 0.245
## 3 Recovery Cantabria      2     26      6.32 0.00578
## 4 Recovery Portugal       2     26      4.83 0.0165
```

```
# Short MMHW
Damage_Stats_SMHW_NO %>%
  levene_test(Dam_Ref_1 ~ Heat_Treatment*Phase*Population)
```

```
## # A tibble: 1 x 4
##   df1    df2 statistic      p
##   <int> <int>    <dbl>    <dbl>
## 1     11    109      5.44 0.000000741
```

```
Damage_Stats_SMHW_NO %>%
  group_by(Phase, Population) %>%
  levene_test(Dam_Ref_1 ~ Heat_Treatment)
```

```
## # A tibble: 4 x 6
##   Phase    Population    df1    df2 statistic      p
##   <fct>    <fct>      <int> <int>    <dbl>    <dbl>
## 1 MHW      Cantabria      2     28      1.80 0.184
## 2 MHW      Portugal       2     28      3.91 0.0318
## 3 Recovery Cantabria      2     27      4.94 0.0149
## 4 Recovery Portugal       2     26      0.129 0.880
```

2.2. Statistical analysis: Mixed model on Aligned Rank Transform Data (ART)

Repeated measures on the same individuals and the lack of independence among replicates was considered by fitting models with random effects (mixed models). For repeated measures, the error associated to each individual (ID) was considered as a random effect while the lack of truly replicates independence was considered by adding Tank as a random factor. Population, MHW intensity and Time were the fixed factors. Then, the structure of the mixed model is as follows: $\text{Damages} \sim \text{Population} * \text{Heat_Treatment} * \text{Phase} + (1 | \text{ID}) + (1 | \text{Tank})$

From the model for the Large MHW what we observed was that there was a statistically significant ($p < .05$) three-way interaction between time, population, and MHW intensity $F[2, 53] = 9.45$, $p < 0.001$. For the short MHW, no significant three-way interaction was detected ($F[2, 54] = 1.63$, $p = 0.206$), but there were significant two-way interactions between Population/Time ($F[1, 54] = 14.40$, $p < 0.001$), MHW intensity/Time ($F[2, 54] = 4.12$, $p = 0.022$) and Population/MHW intensity ($F[2, 56] = 5.89$, $p = 0.005$).

```
# Large MMHW
Damage.art_LRec <- art(Dam_Ref_1 ~ Population*Heat_Treatment*Phase + (1|ID) + (1|Tank) ,
                      data = Damage_Stats_LMHW_NO)
summary(artlm(Damage.art_LRec, c("Population:Heat_Treatment:Phase")))
```



```
## Linear mixed model fit by REML ['lmerMod']
## Formula: .y ~ Population + Heat_Treatment + Phase + (1 | ID) + (1 | Tank) +
##      Population:Heat_Treatment + Population:Phase + Heat_Treatment:Phase +
##      Population:Heat_Treatment:Phase
##      Data: df
##
## REML criterion at convergence: 1084.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.95506 -0.47754 -0.00214  0.54780  1.90101
##
## Random effects:
##      Groups      Name      Variance Std.Dev.
##      ID          (Intercept) 365.3    19.11
##      Tank        (Intercept) 252.5    15.89
##      Residual                647.1    25.44
## Number of obs: 118, groups:  ID, 60; Tank, 6
##
## Fixed effects:
##                                     Estimate Std. Error t value
## (Intercept)                        59.5577     7.3371   8.117
## Population1                         3.8832     3.4519   1.125
## Heat_Treatment1                     2.7508    10.3752   0.265
## Heat_Treatment2                     0.1365    10.3766   0.013
## Phase1                           -0.6437     2.3579  -0.273
## Population1:Heat_Treatment1        -0.3850     4.8925  -0.079
## Population1:Heat_Treatment2       -1.9307     4.8795  -0.396
## Population1:Phase1                 1.4388     2.3579   0.610
## Heat_Treatment1:Phase1            -2.8411     3.3176  -0.856
## Heat_Treatment2:Phase1             2.4715     3.3431   0.739
## Population1:Heat_Treatment1:Phase1 -14.2872     3.3176 -4.306
## Population1:Heat_Treatment2:Phase1  5.3890     3.3431   1.612
##
```

```
## Correlation of Fixed Effects:
##      (Intr) Ppltn1 Ht_Tr1 Ht_Tr2 Phase1 Pp1:H_T1 Pp1:H_T2 Pp1:P1 H_T1:P
## Population1 -0.008
## Het_Trtmnt1  0.000  0.045
## Het_Trtmnt2  0.000 -0.022 -0.500
## Phase1      -0.007 -0.014  0.005 -0.002
## Ppltn1:H_T1  0.045  0.006  0.024 -0.012  0.010
## Ppltn1:H_T2 -0.022 -0.001 -0.012 -0.023 -0.006 -0.503
## Ppltn1:Phs1 -0.007 -0.014  0.005 -0.002 -0.012  0.010 -0.006
## Ht_Trtn1:P1  0.005  0.010 -0.003  0.002 -0.014 -0.007  0.004  0.078
## Ht_Trtn2:P1 -0.002 -0.006  0.002 -0.008  0.007  0.004 -0.018 -0.039 -0.496
## Pp1:H_T1:P1  0.005  0.010 -0.003  0.002  0.078 -0.007  0.004 -0.014  0.043
## Pp1:H_T2:P1 -0.002 -0.006  0.002 -0.008 -0.039  0.004 -0.018  0.007 -0.021
##      H_T2:P P1:H_T1:
## Population1
## Het_Trtmnt1
## Het_Trtmnt2
## Phase1
## Ppltn1:H_T1
## Ppltn1:H_T2
## Ppltn1:Phs1
## Ht_Trtn1:P1
## Ht_Trtn2:P1
## Pp1:H_T1:P1 -0.021
## Pp1:H_T2:P1 -0.040 -0.496
```

```
Damage.aov_LRec <- anova(Damage.art_LRec)
print(Damage.aov_LRec)
```

```
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##              F Df  Df.res    Pr(>F)
## 1 Population          34.231297  1 51.2039 3.4683e-07 ***
## 2 Heat_Treatment        0.081196  2  2.9817 0.92398238
## 3 Phase                54.958079  1 52.8228 9.9373e-10 ***
## 4 Population:Heat_Treatment  5.791433  2 51.0427 0.00541058 **
## 5 Population:Phase        12.897968  1 52.8572 0.00072097 ***
## 6 Heat_Treatment:Phase     9.616275  2 52.8667 0.00027425 ***
## 7 Population:Heat_Treatment:Phase 9.451917  2 52.8504 0.00030957 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Short MMHW
Damage.art_SRec <- art(Dam_Ref_1 ~ Population*Heat_Treatment*Phase + (1|ID) + (1|Tank),
                      data = Damage Stats_SMHW_NO)
summary(artlm(Damage.art_SRec, c("Population:Heat_Treatment:Phase")))
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: .y ~ Population + Heat_Treatment + Phase + (1 | ID) + (1 | Tank) +
```

```

##      Population:Heat_Treatment + Population:Phase + Heat_Treatment:Phase +
##      Population:Heat_Treatment:Phase
##      Data: df
##
## REML criterion at convergence: 1127.7
##
## Scaled residuals:
##      Min      1Q   Median      3Q      Max
## -1.50128 -0.67399 -0.04636  0.51452  1.84269
##
## Random effects:
##      Groups   Name      Variance Std.Dev.
##      ID       (Intercept) 608.4    24.67
##      Tank     (Intercept) 115.4    10.74
##      Residual              638.7    25.27
## Number of obs: 121, groups:  ID, 63; Tank, 6
##
## Fixed effects:
##
##              Estimate Std. Error t value
## (Intercept)      60.5554      5.8685  10.319
## Population1         1.3726      3.9048   0.352
## Heat_Treatment1     2.9159      8.3400   0.350
## Heat_Treatment2    -2.3889      8.2573  -0.289
## Phase1            -0.2564      2.3369  -0.110
## Population1:Heat_Treatment1 -1.2514      5.5791  -0.224
## Population1:Heat_Treatment2  0.8359      5.4548   0.153
## Population1:Phase1    1.8178      2.3364   0.778
## Heat_Treatment1:Phase1  2.1017      3.3550   0.626
## Heat_Treatment2:Phase1  2.0379      3.2500   0.627
## Population1:Heat_Treatment1:Phase1  5.4775      3.3547   1.633
## Population1:Heat_Treatment2:Phase1 -0.4743      3.2496  -0.146
##
## Correlation of Fixed Effects:
##      (Intr) Ppltn1 Ht_Tr1 Ht_Tr2 Phase1 Pp1:H_T1 Pp1:H_T2 Pp1:P1 H_T1:P
## Population1 -0.005
## Het_Trtnmt1  0.014  0.016
## Het_Trtnmt2 -0.014 -0.047 -0.500
## Phase1      -0.016  0.006  0.011 -0.001
## Ppltn1:H_T1  0.016  0.029  0.006  0.024 -0.004
## Ppltn1:H_T2 -0.047 -0.035  0.024 -0.039  0.014 -0.497
## Ppltn1:Phs1  0.005 -0.024 -0.003  0.009  0.006  0.016 -0.002
## Ht_Trtnmt1:P1  0.011 -0.004 -0.008  0.001  0.043  0.003 -0.010  0.057
## Ht_Trtnmt2:P1 -0.001  0.014  0.001 -0.017 -0.047 -0.010  0.016 -0.092 -0.498
## Pp1:H_T1:P1 -0.003  0.016  0.002 -0.006  0.056 -0.011  0.001  0.043  0.045
## Pp1:H_T2:P1  0.009 -0.002 -0.006  0.011 -0.092  0.001 -0.025 -0.047  0.020
##      H_T2:P P1:H_T1:
## Population1
## Het_Trtnmt1
## Het_Trtnmt2
## Phase1
## Ppltn1:H_T1
## Ppltn1:H_T2
## Ppltn1:Phs1
## Ht_Trtnmt1:P1

```

```
## Ht_Trmt2:P1
## Pp1:H_T1:P1 0.020
## Pp1:H_T2:P1 -0.060 -0.499
```

```
Damage.aov_SRec <- anova(Damage.art_SRec)
print(Damage.aov_SRec)
```

```
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##
##              F Df  Df.res      Pr(>F)
## 1 Population      12.42357  1 54.3704 0.00086870 ***
## 2 Heat_Treatment    0.95738  2  2.9633 0.47782138
## 3 Phase             28.48617  1 54.2329 1.9012e-06 ***
## 4 Population:Heat_Treatment  5.88679  2 54.3078 0.00485321 **
## 5 Population:Phase     14.40648  1 54.1520 0.00037377 ***
## 6 Heat_Treatment:Phase    4.11959  2 54.0903 0.02161319 *
## 7 Population:Heat_Treatment:Phase 1.62511  2 54.2312 0.20633118
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.3. Post-hoc analysis

For the Large MHW:

1. Simple two-way interaction analysis: There was only a statistically significant simple two-way interaction between MHW intensity and population for the Recovery period ($F[2, 50] = 6.47$, $p = 0.003$), but not for the MHW end ($F[2, 51] = 0.91$, $p = 0.410$).
2. Simple-simple main effect analysis: There was not a statistically significant simple simple main effect of the MHW intensity on the tissue damages for the Atlantic population after the Recovery ($F[2, 3] = 2.6$, $p = 0.223$) nor for the Cantabrian one ($F[2, 3] = 0.46$, $p = 0.665$).
3. Simple comparisons: No significant differences.

```
# Large MMHW
two.way_LRec <- list()
i=1
for (i in 1:length(unique(Damage_Stats_LMHW_NO$Phase))) {
  a <- Damage_Stats_LMHW_NO[Damage_Stats_LMHW_NO$Phase == as.character(
    unique(Damage_Stats_LMHW_NO$Phase)[i]),]
  b <- art(Dam_Ref_1~ Population*Heat_Treatment + (1|Tank) , data = a)
  c <- anova(b)
  two.way_LRec[[i]] <- c
  names(two.way_LRec)[[i]] <- as.character(unique(Damage_Stats_LMHW_NO$Phase)[i])
  rm(a,b,c)
}
two.way_LRec
```

```

## $MHW
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##
##          F Df Df.res    Pr(>F)
## 1 Population      9.4156  1 51.950 0.0034148 **
## 2 Heat_Treatment    1.1986  2  2.973 0.4152192
## 3 Population:Heat_Treatment 0.9053  2 51.521 0.4107551
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## $Recovery
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##
##          F Df Df.res    Pr(>F)
## 1 Population     23.15250  1 49.8621 1.4263e-05 ***
## 2 Heat_Treatment    0.86053  2  2.9597  0.5074681
## 3 Population:Heat_Treatment 6.47496  2 49.6555  0.0031712 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

MHW.effect_LREC <- list()
i=1
j=1
for (i in 1:2) {
  a <- Damage_Stats_LMHW_NO[Damage_Stats_LMHW_NO$Phase == as.character(
    unique(Damage_Stats_LMHW_NO$Phase)[i]),]
  temp <- list()
  for (j in 1:2) {
    b <- a[a$Population == as.character(unique(a$Population)[j]),]
    c <- art(Dam_Ref_1~ Heat_Treatment + (1|Tank), data = b)
    d <- anova(c)
    temp[[j]] <- d
    names(temp)[[j]] <- paste(as.character(unique(Damage_Stats_LMHW_NO$Phase)[i]),
                             "_",as.character(unique(a$Population)[j]))
    rm(b,c,d)
  }
  MHW.effect_LREC[[i]] <- temp
  rm(temp)
}
MHW.effect_LREC

```

```

## [[1]]
## [[1]]$'MHW _ Cantabria'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)

```

```

## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##           F Df Df.res  Pr(>F)
## 1 Heat_Treatment 4.036  2 2.6326 0.15781
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## [[1]]$'MHW _ Portugal'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##           F Df Df.res  Pr(>F)
## 1 Heat_Treatment 0.15826  2 2.9867 0.86034
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## [[2]]
## [[2]]$'Recovery _ Cantabria'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##           F Df Df.res  Pr(>F)
## 1 Heat_Treatment 0.46904  2  2.948 0.66549
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## [[2]]$'Recovery _ Portugal'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##           F Df Df.res  Pr(>F)
## 1 Heat_Treatment 2.5897  2 2.9735 0.22322
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

pwc_LRec <- list()
i=1
j=1
for (i in 1:2) {
  a <- Damage_Stats_LMHW_NO[Damage_Stats_LMHW_NO$Phase == as.character(
    unique(Damage_Stats_LMHW_NO$Phase)[i]),]
  temp <- list()
  for (j in 1:2) {

```


2. Simple-simple main effect analysis: There was not a statistically significant simple simple main effect of the MHW intensity on the tissue damages for any population and measuring time.
3. Simple comparisons: No significant differences detected.

```
# Short MHW
MHW.effect_SRec <- list()
i=1
j=1
for (i in 1:2) {
  a <- Damage_Stats_SMHW_NO[Damage_Stats_SMHW_NO$Phase == as.character(
    unique(Damage_Stats_SMHW_NO$Phase)[i]),]
  temp <- list()
  for (j in 1:2) {
    b <- a[a$Population == as.character(unique(a$Population)[j]),]
    c <- art(Dam_Ref_1~ Heat_Treatment + (1|Tank), data = b)
    d <- anova(c)
    temp[[j]] <- d
    names(temp)[[j]] <- paste(as.character(unique(Damage_Stats_SMHW_NO$Phase)[i]),
                              "_",as.character(unique(a$Population)[j]))
    rm(b,c,d)
  }
  MHW.effect_SRec[[i]] <- temp
  rm(temp)
}
MHW.effect_SRec

## [[1]]
## [[1]]$'MHW _ Cantabria'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##              F Df Df.res Pr(>F)
## 1 Heat_Treatment 1.8384  2  2.881 0.3058
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## [[1]]$'MHW _ Portugal'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##              F Df Df.res  Pr(>F)
## 1 Heat_Treatment 0.6177  2 2.9239 0.59736
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##
## [[2]]
```

```
## [[2]]$'Recovery _ Cantabria'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##               F Df Df.res  Pr(>F)
## 1 Heat_Treatment 1.6272  2 2.6985 0.34386
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## [[2]]$'Recovery _ Portugal'
## Analysis of Variance of Aligned Rank Transformed Data
##
## Table Type: Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df)
## Model: Mixed Effects (lmer)
## Response: art(Dam_Ref_1)
##
##               F Df Df.res  Pr(>F)
## 1 Heat_Treatment 1.2747  2 2.6389 0.40983
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pwc_SRec <- list()
i=1
j=1
for (i in 1:2) {
  a <- Damage_Stats_SMHW_NO[Damage_Stats_SMHW_NO$Phase == as.character(
    unique(Damage_Stats_SMHW_NO$Phase)[i]),]
  temp <- list()
  for (j in 1:2) {
    b <- a[a$Population == as.character(unique(a$Population)[j]),]
    c <- art(Dam_Ref_1~ Heat_Treatment, data = b)
    d <- art.con(c, "Heat_Treatment")
    d <- as.data.frame(d)
    d$Phase <- as.character(unique(Damage_Stats_SMHW_NO$Phase)[i])
    d$Population <- as.character(unique(a$Population)[j])
    temp[[j]] <- d
    rm(b,c,d)
  }
  temp <- do.call(rbind, temp)
  pwc_SRec[[i]] <- temp
  rm(temp)
}

pwc_SRec <- as.data.frame(do.call(rbind, pwc_SRec))
pwc_SRec$Duration_Treatment <- "Srec"
pwc_SRec
```

## contrast	estimate	SE	df	t.ratio	p.value	Phase	Population
## Control - MHW2	-6.736111	3.865222	28	-1.743	0.2075	MHW	Cantabria
## Control - MHW4	-1.044444	4.027469	28	-0.259	0.9637	MHW	Cantabria
## MHW2 - MHW4	5.691667	3.753163	28	1.516	0.2987	MHW	Cantabria

```

## Control - MHW2 -0.950000 4.069197 28 -0.233 0.9704 MHW Portugal
## Control - MHW4 -4.913636 3.975639 28 -1.236 0.4425 MHW Portugal
## MHW2 - MHW4 -3.963636 3.975639 28 -0.997 0.5848 MHW Portugal
## Control - MHW2 -4.833333 3.634146 27 -1.330 0.3913 Recovery Cantabria
## Control - MHW4 1.444444 3.885066 27 0.372 0.9268 Recovery Cantabria
## MHW2 - MHW4 6.277778 3.634146 27 1.727 0.2135 Recovery Cantabria
## Control - MHW2 1.033333 3.810775 26 0.271 0.9603 Recovery Portugal
## Control - MHW4 -4.700000 3.709136 26 -1.267 0.4259 Recovery Portugal
## MHW2 - MHW4 -5.733333 3.810775 26 -1.505 0.3053 Recovery Portugal
## Duration_Treatment
## Srec
## Srec
## Srec
## Srec
## Srec
## Srec
## Srec
## Srec
## Srec
## Srec
## Srec
## Srec
## P value adjustment: tukey method for comparing a family of 3 estimates

```