The Rice example: illustrating the first five steps for smoothing and extracting traits (SET) using growthPheno

Chris Brien

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This example is based on the data whose analysis has been published by Al-Tamimi et al. (2016). The five steps of the method for smoothing and extracting traits (SET) described in detail in Brien et al. (2020) is illustrated for this data.

Initialize

Step 1: Import, select and derive longitudinal data

Step 1(a): Import the data

```
data(RiceRaw.dat)
```

Step 1(b): Organize the data

Here the imaging variables are selected and covariates and factors added to produce `longi.dat`.

```
longi.dat <- longitudinalPrime(data=RiceRaw.dat, smarthouse.lev=c("NE","NW"))

longi.dat <- designFactors(longi.dat, insertName = "xDays",
                           designfactorMethod="StandardOrder")

# Particular edits to longi.dat
longi.dat <- within(longi.dat,
                    {
                      Days.after.Salting <- as.numfac(Days) - 29
                    })
longi.dat <- with(longi.dat, longi.dat[order(Snapshot.ID.Tag,Days), ])
```

Step 1(c): Derive longitudinal traits that result in a value for each observation

```
# Set responses
responses.image <- c("Area")
responses.smooth <- paste(responses.image, "smooth", sep=".")

# Form growth rates for each observation of a subset of responses by differencing
longi.dat <- splitContGRdiff(longi.dat, responses.image,
                             INDICES="Snapshot.ID.Tag",
                             which.rates = c("AGR","RGR"))
```

```
# Form Area.WUI
longi.dat <- within(longi.dat,
                    {
                      Area.WUI <- WUI(Area.AGR*Days.diffs, Water.Loss)
                    })

# Add cumulative responses
longi.dat <- within(longi.dat,
                    {
                      Water.Loss.Cum <- unlist(by(Water.Loss, Snapshot.ID.Tag,
                                                  cumulate, exclude.1st=TRUE))
                      WUI.cum <- Area / Water.Loss.Cum
                    })
# Check longi.dat
head(longi.dat)
```

```
##   Snapshot.ID.Tag Days Smarthouse Lane Position Snapshot.Time.Stamp xPosn Reps
## 1         045451-C   28         NE    1        2 2015-02-18 02:14:00   -11    1
## 2         045451-C   30         NE    1        2 2015-02-20 02:14:00   -11    1
## 3         045451-C   31         NE    1        2 2015-02-21 02:14:00   -11    1
## 4         045451-C   32         NE    1        2 2015-02-22 02:14:00   -11    1
## 5         045451-C   33         NE    1        2 2015-02-23 02:14:00   -11    1
## 6         045451-C   34         NE    1        2 2015-02-24 02:14:00   -11    1
##       Hour     xDays Zones xZones SHZones ZLane ZMainplots Subplots xMainPosn
## 1 2.233333 -7.428571     1   -2.5       1     1          1        1     -10.5
## 2 2.233333 -5.428571     1   -2.5       1     1          1        1     -10.5
## 3 2.233333 -4.428571     1   -2.5       1     1          1        1     -10.5
## 4 2.233333 -3.428571     1   -2.5       1     1          1        1     -10.5
## 5 2.233333 -2.428571     1   -2.5       1     1          1        1     -10.5
## 6 2.233333 -1.428571     1   -2.5       1     1          1        1     -10.5
##   Genotype.ID Treatment.1 Weight.Before Weight.After Water.Amount Water.Loss
## 1      121080     Control          4007         4031           28         NA
## 2      121080     Control          4056         4084           32        -25
## 3      121080     Control          4036         4083           52         48
## 4      121080     Control          4027         4085           61         56
## 5      121080     Control          4019         4084           69         66
## 6      121080     Control          4014         4083           74         70
##      Area Area.SV1 Area.SV2 Area.TV Boundary.Points.To.Area.Ratio.SV1
## 1  57.446   20.912   11.526  25.008                           0.353912
## 2  89.306   29.073   21.495  38.738                           0.310735
## 3 100.138   27.751   26.835  45.552                           0.354293
## 4 128.323   34.697   32.848  60.778                           0.371012
## 5 158.776   46.779   37.871  74.126                           0.319823
## 6 182.551   48.849   48.794  84.908                           0.328400
##   Boundary.Points.To.Area.Ratio.SV2 Boundary.Points.To.Area.Ratio.TV
## 1                          0.454104                          0.197537
## 2                          0.401396                          0.172182
## 3                          0.332364                          0.174175
## 4                          0.358469                          0.178157
## 5                          0.347179                          0.172517
## 6                          0.290220                          0.163153
##   Caliper.Length.SV1 Caliper.Length.SV2 Caliper.Length.TV Compactness.SV1
## 1            666.013            668.692           704.189       0.0930821
## 2            632.735            729.044           830.812       0.1327200
```

```
## 3            731.077            931.028          1104.350       0.0925419
## 4            791.760            878.427          1029.300       0.0969068
## 5            830.360            965.221          1197.530       0.1241550
## 6           1103.050            991.259          1408.310       0.0938637
##   Compactness.SV2 Compactness.TV Convex.Hull.Area.SV1 Convex.Hull.Area.SV2
## 1       0.0689923      0.1435880              224.662               167.062
## 2       0.0734412      0.1091450              219.055               292.683
## 3       0.0678337      0.0950009              299.875               395.600
## 4       0.0707469      0.1102850              358.045               464.303
## 5       0.0783589      0.1119250              376.780               483.302
## 6       0.1014870      0.0947390              520.425               480.792
##   Convex.Hull.Area.TV Center.Of.Mass.Y.SV1 Center.Of.Mass.Y.SV2
## 1             174.165              1841.78              1788.86
## 2             354.921              1837.62              1797.42
## 3             479.490              1826.88              1757.60
## 4             551.097              1798.03              1750.54
## 5             662.283              1796.70              1781.50
## 6             896.231              1809.42              1778.94
##   Max.Dist.Above.Horizon.Line.SV1 Max.Dist.Above.Horizon.Line.SV2
## 1                             620                             624
## 2                             543                             541
## 3                             642                             633
## 4                             736                             823
## 5                             658                             652
## 6                             639                             633
##   Days.after.Salting Days.diffs Area.AGR  Area.RGR   Area.WUI    WUI.cum
## 1                 -1         NA       NA        NA         NA         NA
## 2                  1          2   15.930 0.2206116 -1.2744000 -3.5722400
## 3                  2          1   10.832 0.1144806  0.2256667  4.3538261
## 4                  3          1   28.185 0.2480013  0.5033036  1.6243418
## 5                  4          1   30.453 0.2129439  0.4614091  1.0950069
## 6                  5          1   23.775 0.1395352  0.3396429  0.8490744
##   Water.Loss.Cum
## 1             NA
## 2            -25
## 3             23
## 4             79
## 5            145
## 6            215
```

Step 2: Exploratory analysis

Step 2(a): Fit splines to smooth the longitudinal trends in the primary traits and calculate their growth rates

The `smoothing.method` used is `direct` and `df` is set to 4. The growth rates are calculated by difference, rather than from the spline derivatives.

```
# Smooth responses
for (response in c(responses.image, "Water.Loss"))
  longi.dat <- splitSplines(longi.dat, response, x="xDays", INDICES = "Snapshot.ID.Tag",
                            df = 4, na.x.action="exclude", na.y.action = "exclude")
```

```
## Warning in FUN(X[[i]], ...): Need at least 4 distinct x values to fit a spline -
```

```
## all fitted values set to NA

## Warning in FUN(X[[i]], ...): Need at least 4 distinct x values to fit a spline -
## all fitted values set to NA

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## Warning in FUN(X[[i]], ...): Need at least 4 distinct x values to fit a spline -
```

```
## all fitted values set to NA

## Warning in FUN(X[[i]], ...): Need at least 4 distinct x values to fit a spline -
## all fitted values set to NA

## Warning in FUN(X[[i]], ...): Need at least 4 distinct x values to fit a spline -
## all fitted values set to NA

## Warning in FUN(X[[i]], ...): Need at least 4 distinct x values to fit a spline -
## all fitted values set to NA
```

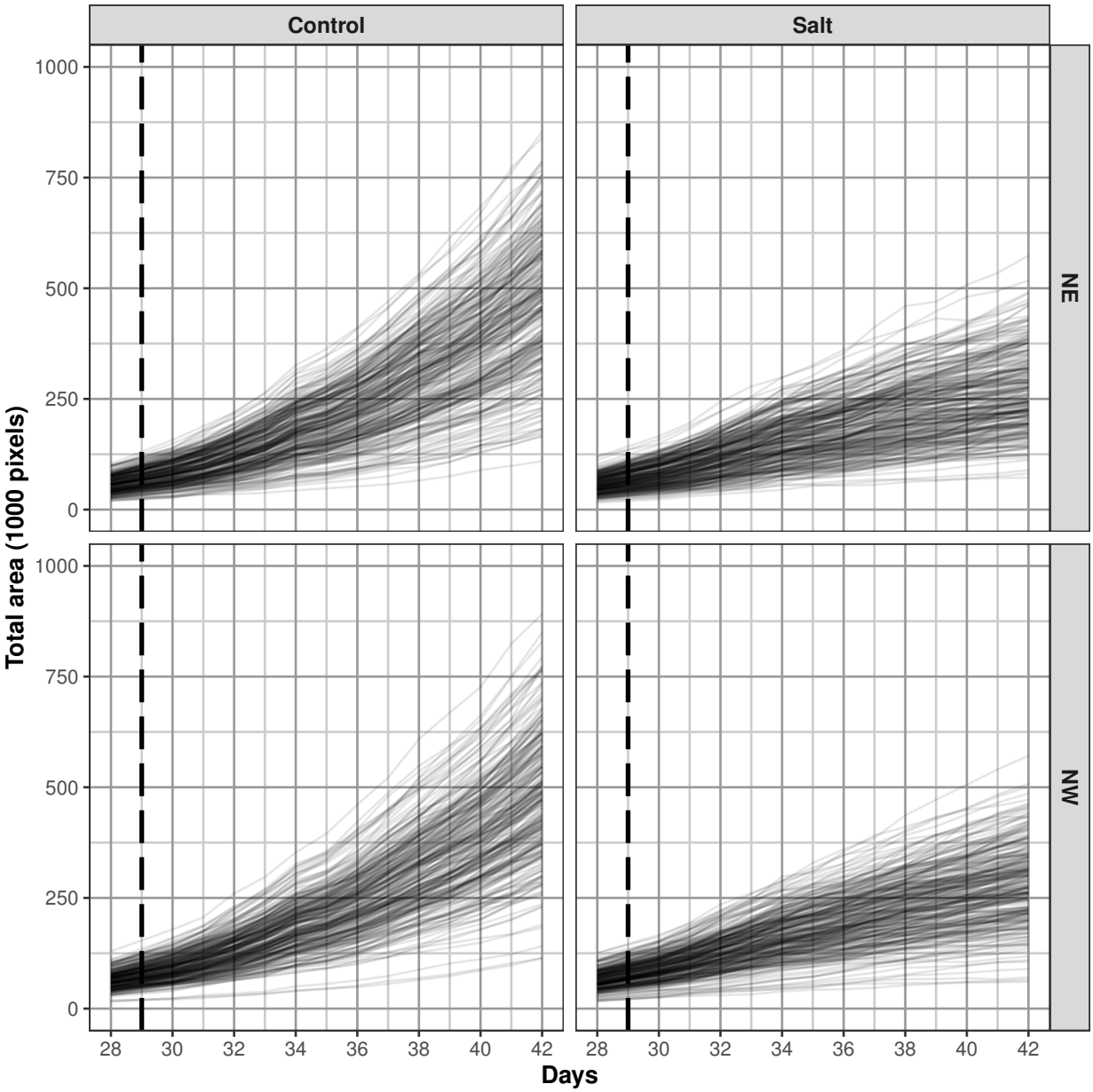
```
longi.dat <- with(longi.dat, longi.dat[order(Snapshot.ID.Tag, xDays), ])

# Loop over smoothed responses, forming growth rates by differences
responses.GR <- paste(responses.smooth, "AGR", sep=".")
longi.dat <- splitContGRdiff(longi.dat, responses.smooth,
                             INDICES="Snapshot.ID.Tag",
                             which.rates = c("AGR","RGR"))

# Finalize longi.dat
longi.dat <- with(longi.dat, longi.dat[order(Snapshot.ID.Tag, xDays), ])
```

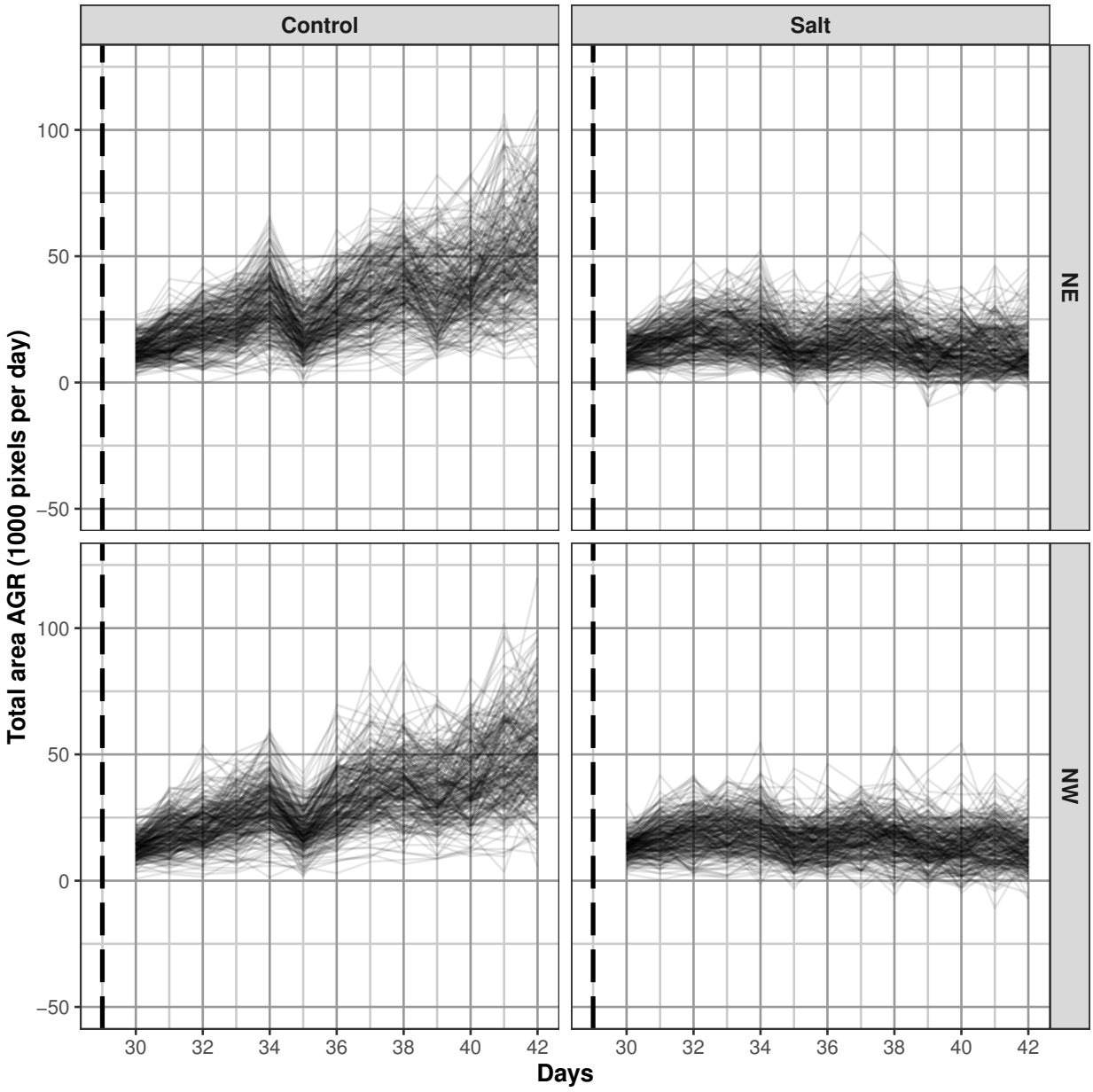
Step 2(b): Compare plots of unsmoothed and smoothed longitudinal data

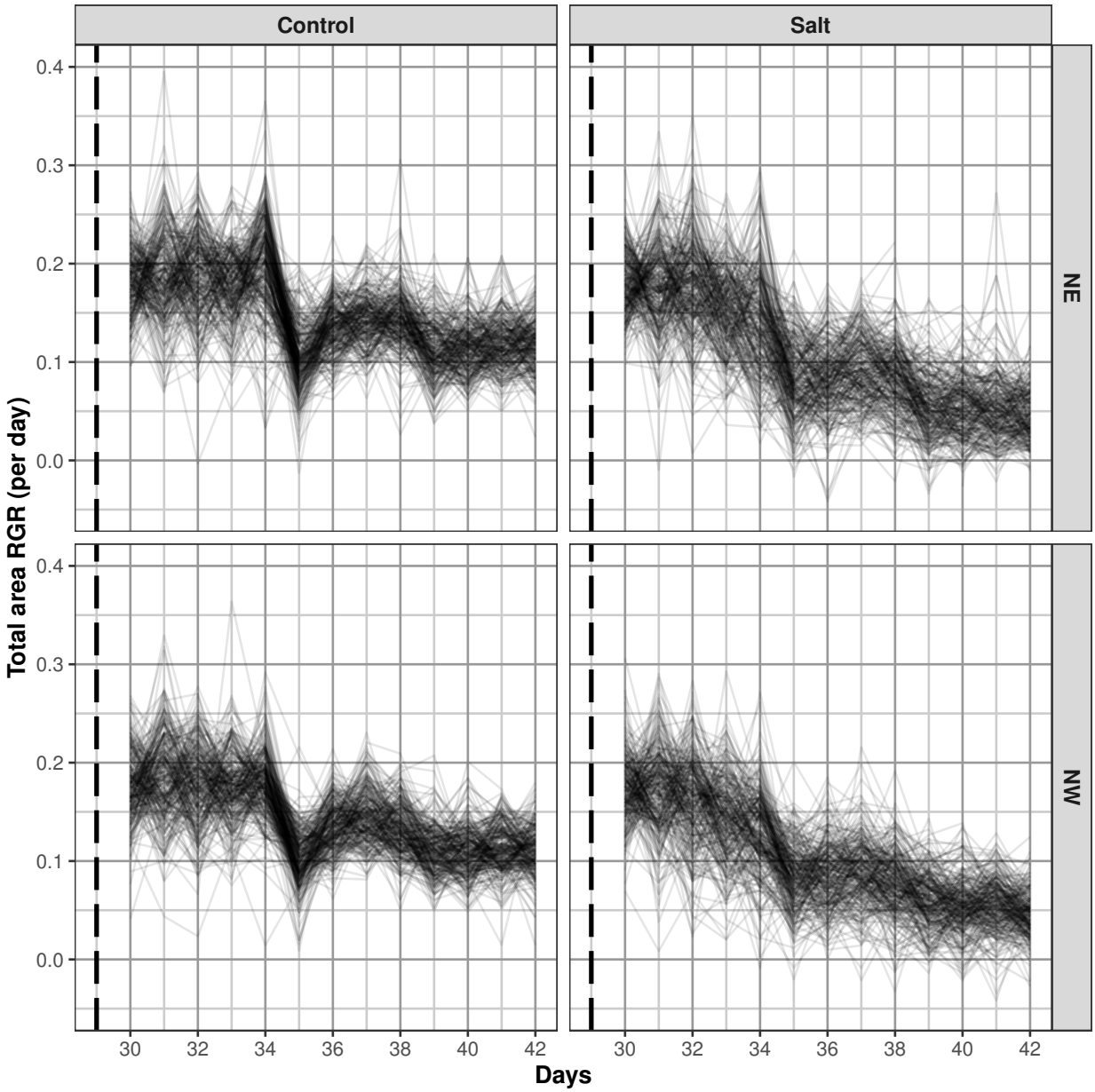
```
responses.longi <- c("Area","Area.AGR","Area.RGR", "Area.WUI")
responses.smooth.plot <- c("Area.smooth","Area.smooth.AGR","Area.smooth.RGR")
titles <- c("Total area (1000 pixels)",
            "Total area AGR (1000 pixels per day)", "Total area RGR (per day)",
            "Total area WUI (1000 pixels per mL)")
titles.smooth<-titles
nresp <- length(responses.longi)
limits <- list(c(0,1000), c(-50,125), c(-0.05,0.40), c(0,30))
```

Plot unsmoothed profiles for all longitudinal responses

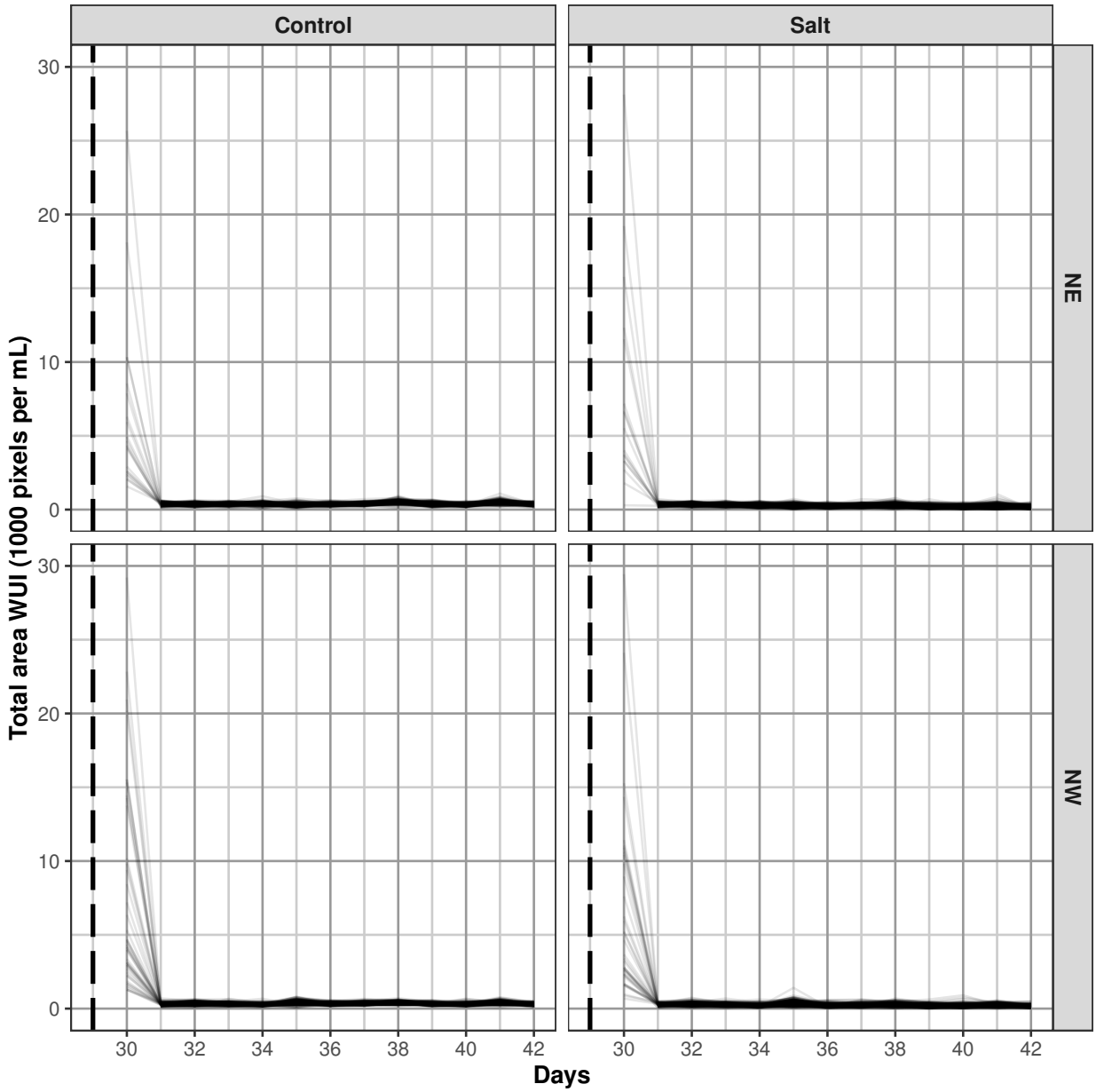
```
klimit <- 0
for (k in 1:nresp)
{
  klimit <- klimit + 1
  plt <- plotLongitudinal(data = longi.dat, response = responses.longi[k],
                          y.title = titles[k], x="xDays+35.42857143", printPlot=FALSE)
  plt <- plt + geom_vline(xintercept=29, linetype="longdash", size=1) +
               scale_x_continuous(breaks=seq(28, 42, by=2)) +
               scale_y_continuous(limits=limits[[klimit]])
  print(plt)
}
```


Control
Salt
NE
NW
Total area (1000 pixels)
0
250
500
750
1000
28
30
32
34
36
38
40
42
Days


Control
Salt
NE
NW
Total area AGR (1000 pixels per day)
100
50
0
−50
30
32
34
36
38
40
42
Days

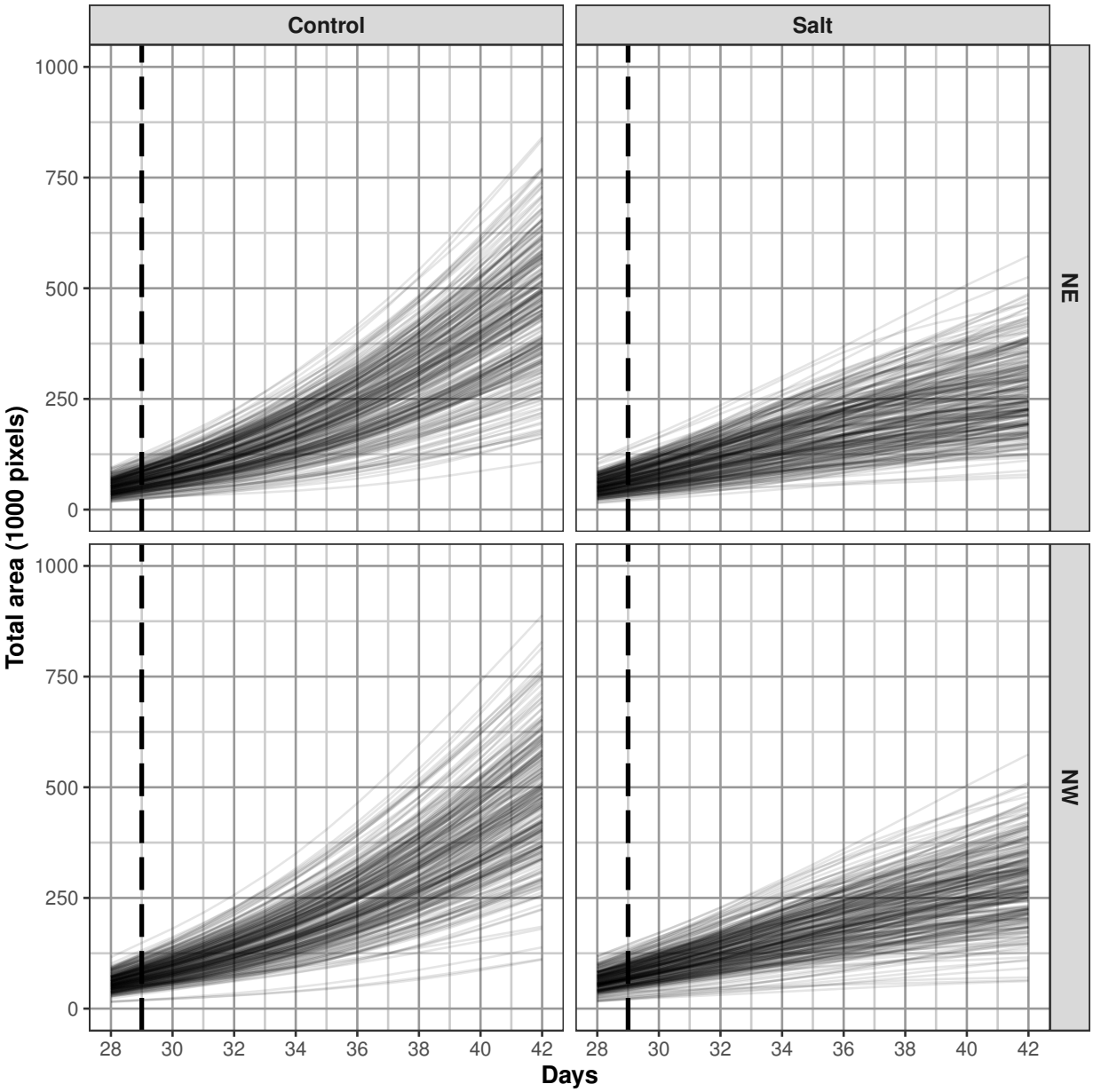


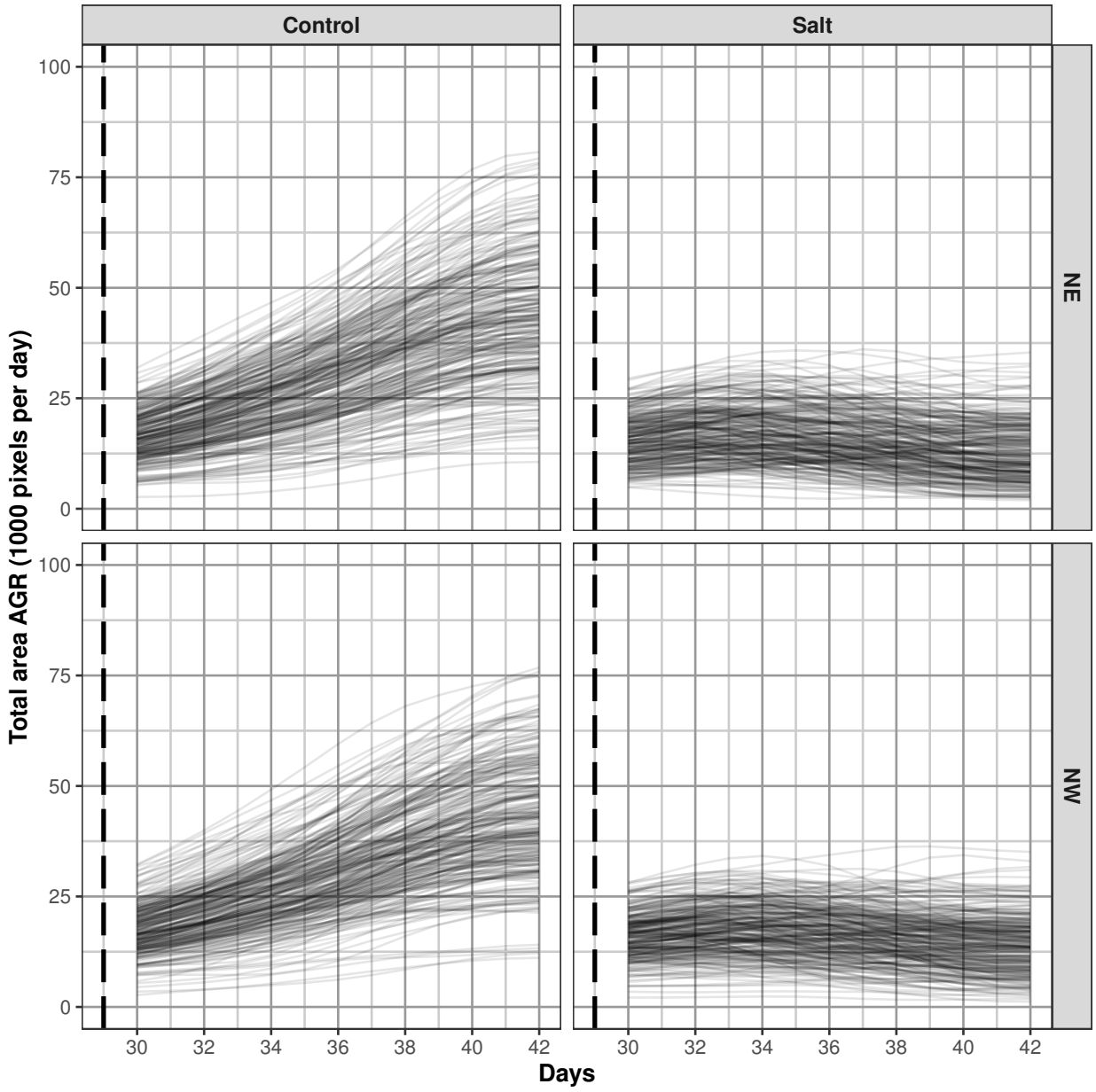

```
## Warning: Removed 932 rows containing missing values (geom_path).
```

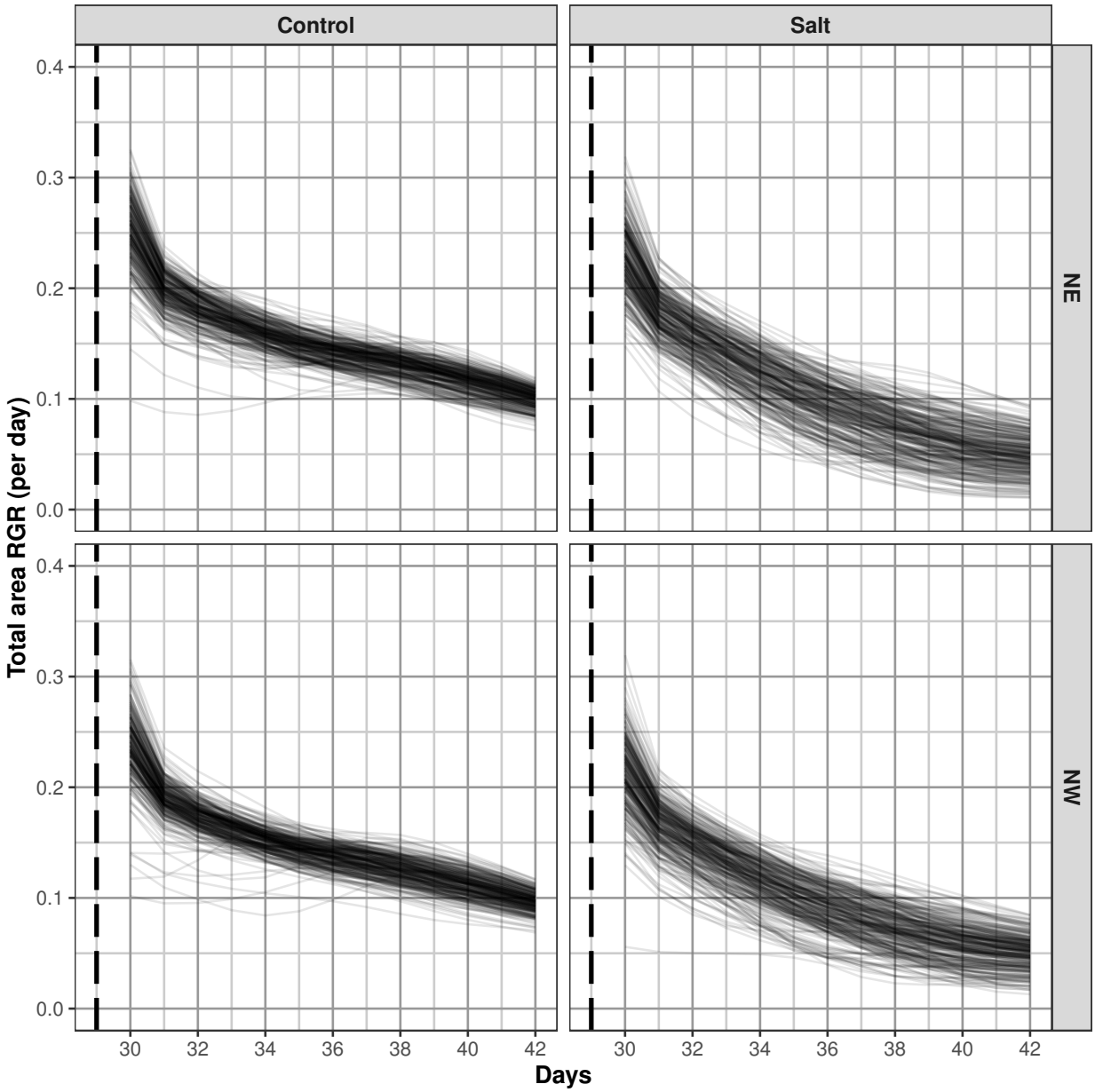


Plot smoothed profiles for all longitudinal responses

```
nresp.smooth <- length(responses.smooth.plot)
limits <- list(c(0,1000), c(0,100), c(0.0,0.40))
for (k in 1:nresp.smooth)
{
  plt <- plotLongitudinal(data = longi.dat, response = responses.smooth.plot[k],
                          y.title = titles.smooth[k], x="xDays+35.42857143", printPlot=FALSE)
  plt <- plt + geom_vline(xintercept=29, linetype="longdash", size=1) +
               scale_x_continuous(breaks=seq(28, 42, by=2)) +
               scale_y_continuous(limits=limits[[k]])
  print(plt)
}
```


Control
Salt
NE
NW
Total area (1000 pixels)
0
250
500
750
1000
28
30
32
34
36
38
40
42
Days


Control
Salt
NE
NW
Total area AGR (1000 pixels per day)
Days
100
75
50
25
0
30
32
34
36
38
40
42



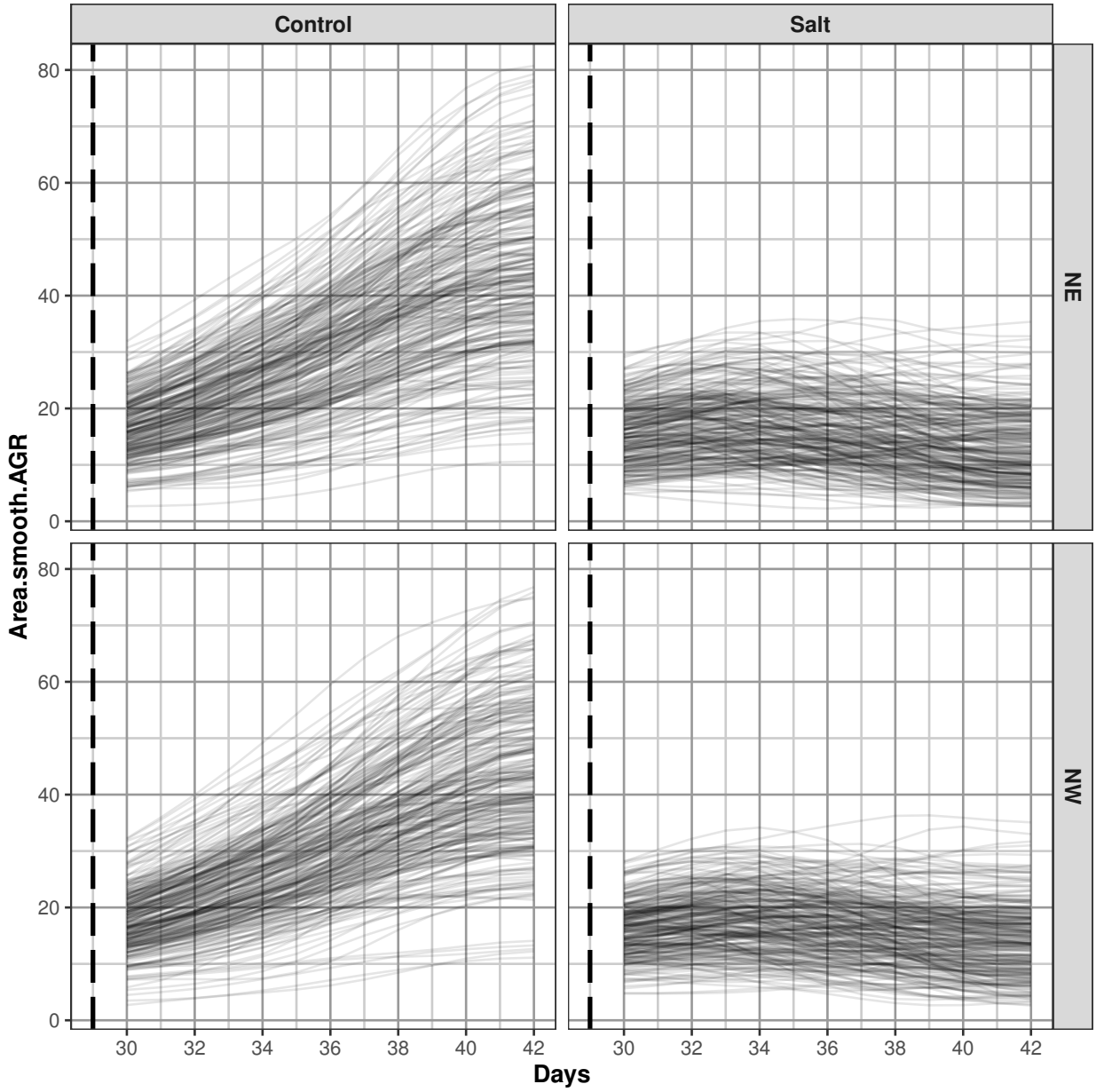
Step 3: Choose the smoothing method and DF

This step has been omitted.

Step 4: Identify potential outlers and clean the data

It has been decided that plants whose smoothed AGR are less than 2.5 after Day 40 are growing so slowly as to be considered anomalous. These plants are identified using `plotAnom`. Their values on Day 42 are printed. The plants are plotted without the anomalous plants followed by a plot of just the anomalous plants. The images of these anomlaous plants were examined and no particular problems were identified with them. They were retained in the data.

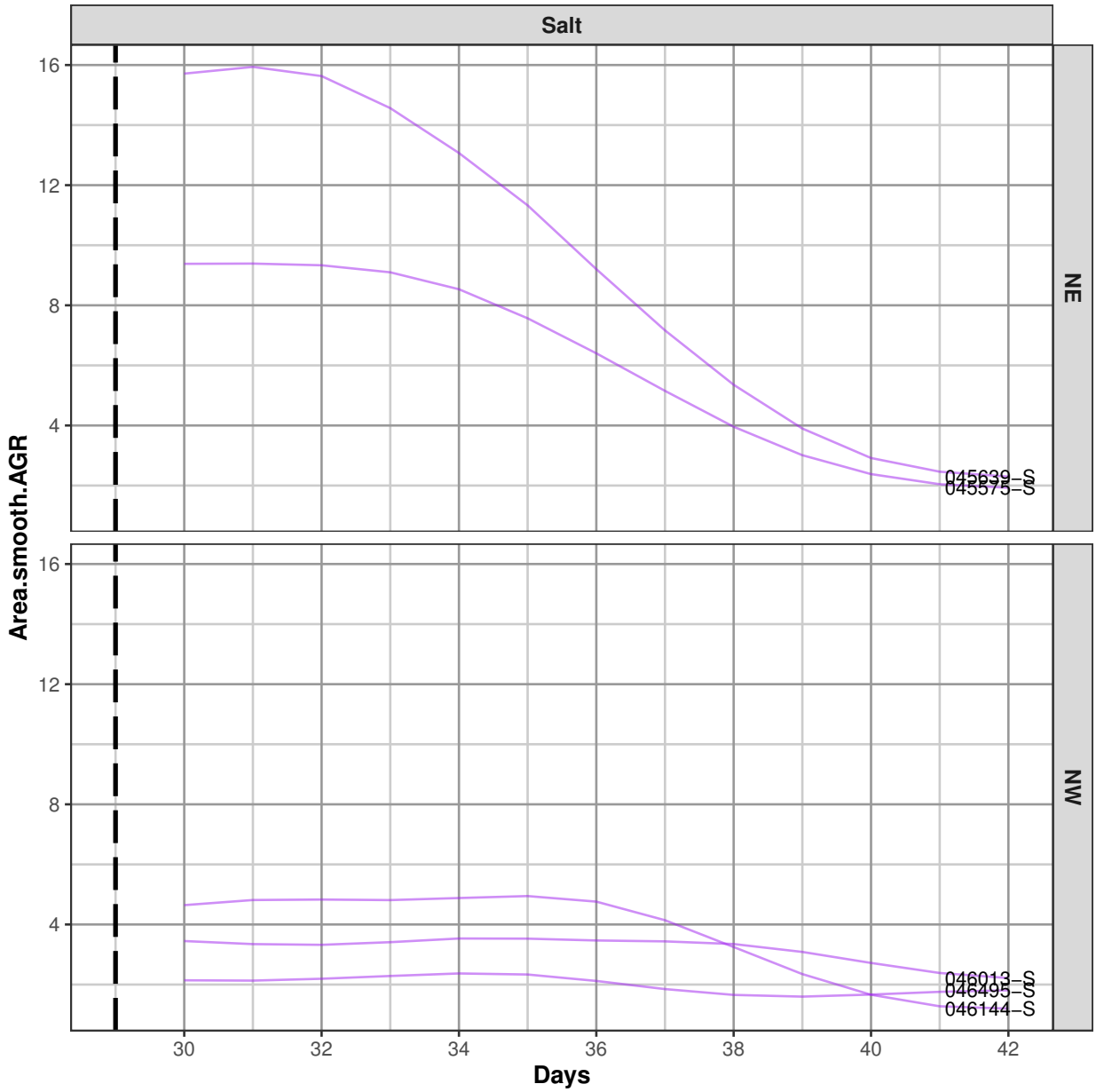
```
anom.ID <- vector(mode = "character", length = 0L)
response <- "Area.smooth.AGR"
cols.output <- c("Snapshot.ID.Tag", "Smarthouse", "Lane", "Position",
                 "Treatment.1", "Genotype.ID", "Days")
anomalous <- plotAnom(longi.dat, response=response, lower=2.5, start.time=40,
                      x = "xDays+35.42857143", vertical.line=29, breaks=seq(28, 42, by=2),
                      whichPrint=c("innerPlot"), y.title=response)
```




```
subs <- subset(anomalous$data, Area.smooth.AGR.anom & Days==42)
if (nrow(subs) == 0)
{ cat("\n#### No anomalous data here\n\n")
} else
{
  subs <- subs[order(subs["Smarthouse"],subs["Treatment.1"], subs[response]),]
```

```
  print(subs[c(cols.output, response)])
  anom.ID <- unique(c(anom.ID, subs$Snapshot.ID.Tag))
  outerPlot <- anomalous$outerPlot  + geom_text(data=subs,
                                                aes_string(x = "xDays+35.42857143",
                                                           y = response,
                                                           label="Snapshot.ID.Tag"),
                                                size=3, hjust=0.7, vjust=0.5)
  print(outerPlot)
}
```

```
##       Snapshot.ID.Tag Smarthouse Lane Position Treatment.1 Genotype.ID Days
## 1680          045575-S         NE    6       10        Salt      121701   42
## 2534          045639-S         NE    9        6        Salt      122000   42
## 9282          046144-S         NW    7        5        Salt      121133   42
## 14000         046495-S         NW   22       10        Salt      120952   42
## 7532          046013-S         NW    1       12        Salt      121852   42
##       Area.smooth.AGR
## 1680         1.926575
## 2534         2.297119
## 9282         1.199223
## 14000        1.809133
## 7532         2.216099
```



Step 5: Extract per-cart traits

A range of single-value plant responses are formed in `Snapshot.ID.Tag` order.

Step 5(a): Set up a data frame with factors only

```
cart.dat <- longi.dat[longi.dat$Days == 31,
                      c("Smarthouse","Lane","Position","Snapshot.ID.Tag",
                        "xPosn","xMainPosn",
                        "Zones","xZones","SHZones","ZLane","ZMainplots", "Subplots",
                        "Genotype.ID","Treatment.1")]
cart.dat <- cart.dat[do.call(order, cart.dat), ]
```

Step 5(b): Get responses based on first and last date.

```
# Observation for first and last date
cart.dat <- cbind(cart.dat, getTimesSubset(responses.image, data = longi.dat,
                                           which.times = c(31), suffix = "first"))
cart.dat <- cbind(cart.dat, getTimesSubset(responses.image, data = longi.dat,
                                           which.times = c(42), suffix = "last"))
cart.dat <- cbind(cart.dat, getTimesSubset(c("WUI.cum"),
                                           data = longi.dat,
                                           which.times = c(42), suffix = "last"))
responses.smooth <- paste(responses.image, "smooth", sep=".")
cart.dat <- cbind(cart.dat, getTimesSubset(responses.smooth, data = longi.dat,
                                           which.times = c(31), suffix = "first"))
cart.dat <- cbind(cart.dat, getTimesSubset(responses.smooth, data = longi.dat,
                                           which.times = c(42), suffix = "last"))

# Growth rates over whole period.
tottime <- 42 - 31
cart.dat <- within(cart.dat,
                   {
                     Area.AGR <- (Area.last - Area.first)/tottime
                     Area.RGR <- log(Area.last / Area.first)/tottime
                   })

# Calculate water index over whole period
cart.dat <- merge(cart.dat,
                  intervalWUI("Area", water.use = "Water.Loss",
                              start.times = c(31),
                              end.times = c(42),
                              suffix = NULL,
                              data = longi.dat, include.total.water = TRUE),
                  by = c("Snapshot.ID.Tag"))
names(cart.dat)[match(c("Area.WUI","Water.Loss.Total"),names(cart.dat))] <-
  c("Area.Overall.WUI", "Water.Loss.Overall")
cart.dat$Water.Loss.rate.Overall <- cart.dat$Water.Loss.Overall / (42 - 31)
```

Step 5(c): Add growth rates and water indices for intervals

```
# Set up intervals
start.days <- list(31,35,31,38)
end.days <- list(35,38,38,42)
suffices <- list("31to35","35to38","31to38","38to42")

# Growth rates for specific intervals from the smoothed data by differencing
for (r in responses.smooth)
{ for (k in 1:length(suffices))
  {
    cart.dat <- merge(cart.dat,
                      intervalGRdiff(r,
                                     which.rates = c("AGR","RGR"),
                                     start.times = start.days[k][[1]],
                                     end.times = end.days[k][[1]],
                                     suffix.interval = suffices[k][[1]],
```

```
                                    data = longi.dat),
                      by = "Snapshot.ID.Tag")
  }
}

# Water indices for specific intervals from the unsmoothed and smoothed data
for (k in 1:length(suffices))
{
  cart.dat <- merge(cart.dat,
                    intervalWUI("Area", water.use = "Water.Loss",
                                start.times = start.days[k][[1]],
                                end.times = end.days[k][[1]],
                                suffix = suffices[k][[1]],
                                data = longi.dat, include.total.water = TRUE),
                    by = "Snapshot.ID.Tag")
  names(cart.dat)[match(paste("Area.WUI", suffices[k][[1]], sep="."),
                        names(cart.dat))] <- paste("Area.WUI", suffices[k][[1]], sep=".")
  cart.dat[paste("Water.Loss.rate", suffices[k][[1]], sep=".")] <-
           cart.dat[[paste("Water.Loss.Total", suffices[k][[1]], sep=".")]] /
                                              ( end.days[k][[1]] - start.days[k][[1]])
}

cart.dat <- with(cart.dat, cart.dat[order(Snapshot.ID.Tag), ])
```

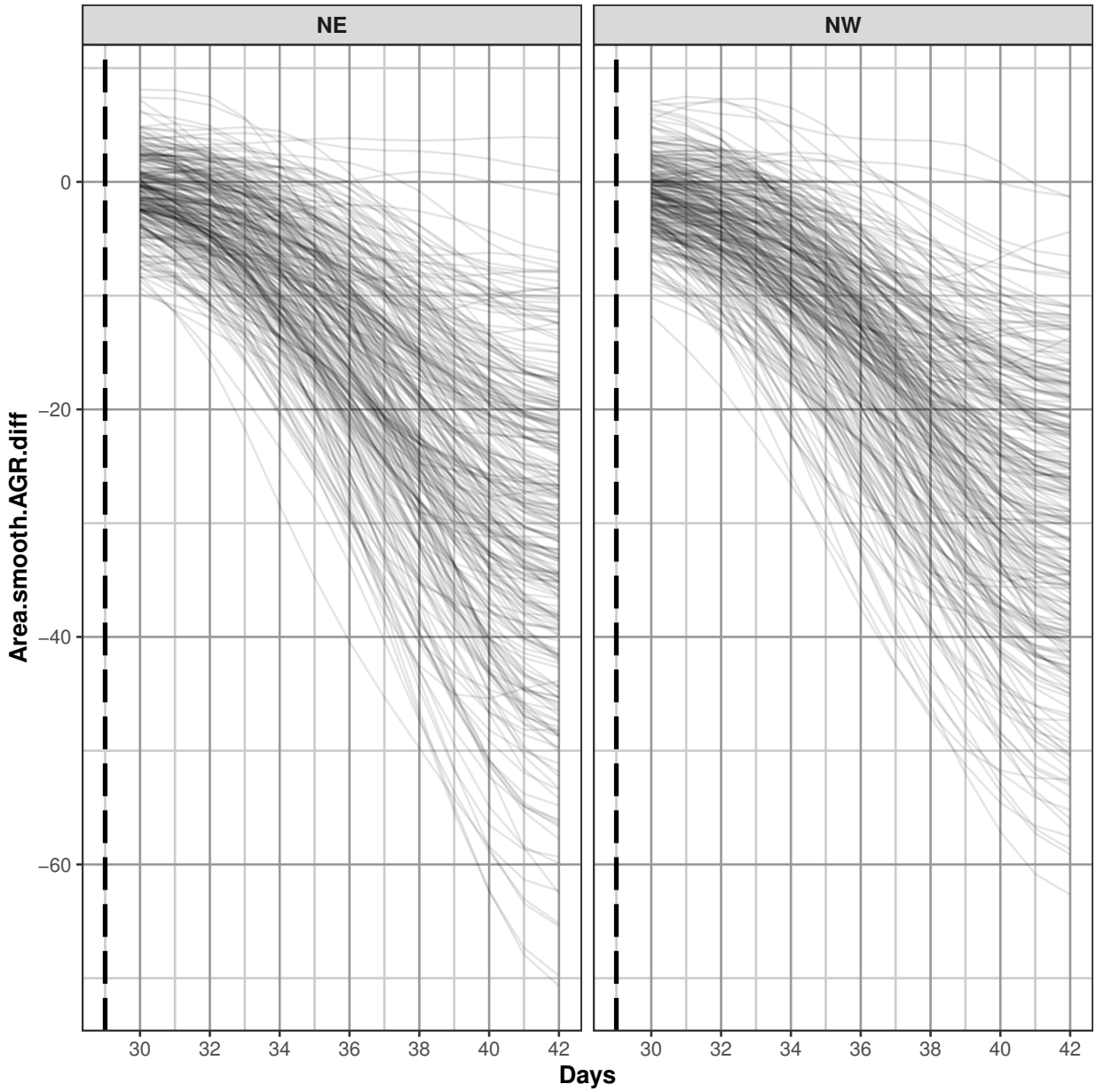
Form continuous and interval SIITs

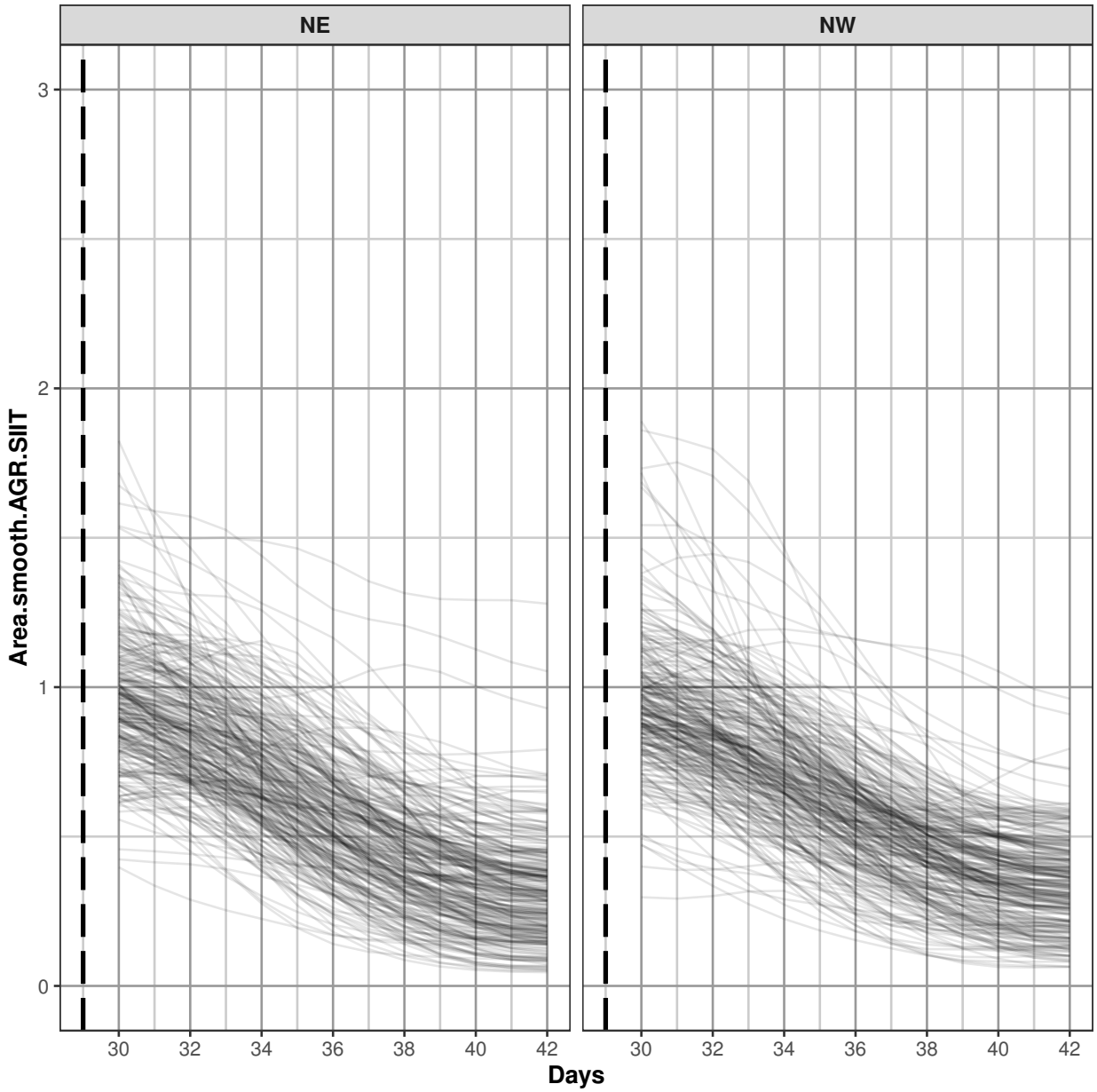
This experiment involved the extra step of calculating a measure of shoot ion-independent tolerance (SIIT) of pairs of plants, control and a salt-treated co-located plants.

Calculate continuous values

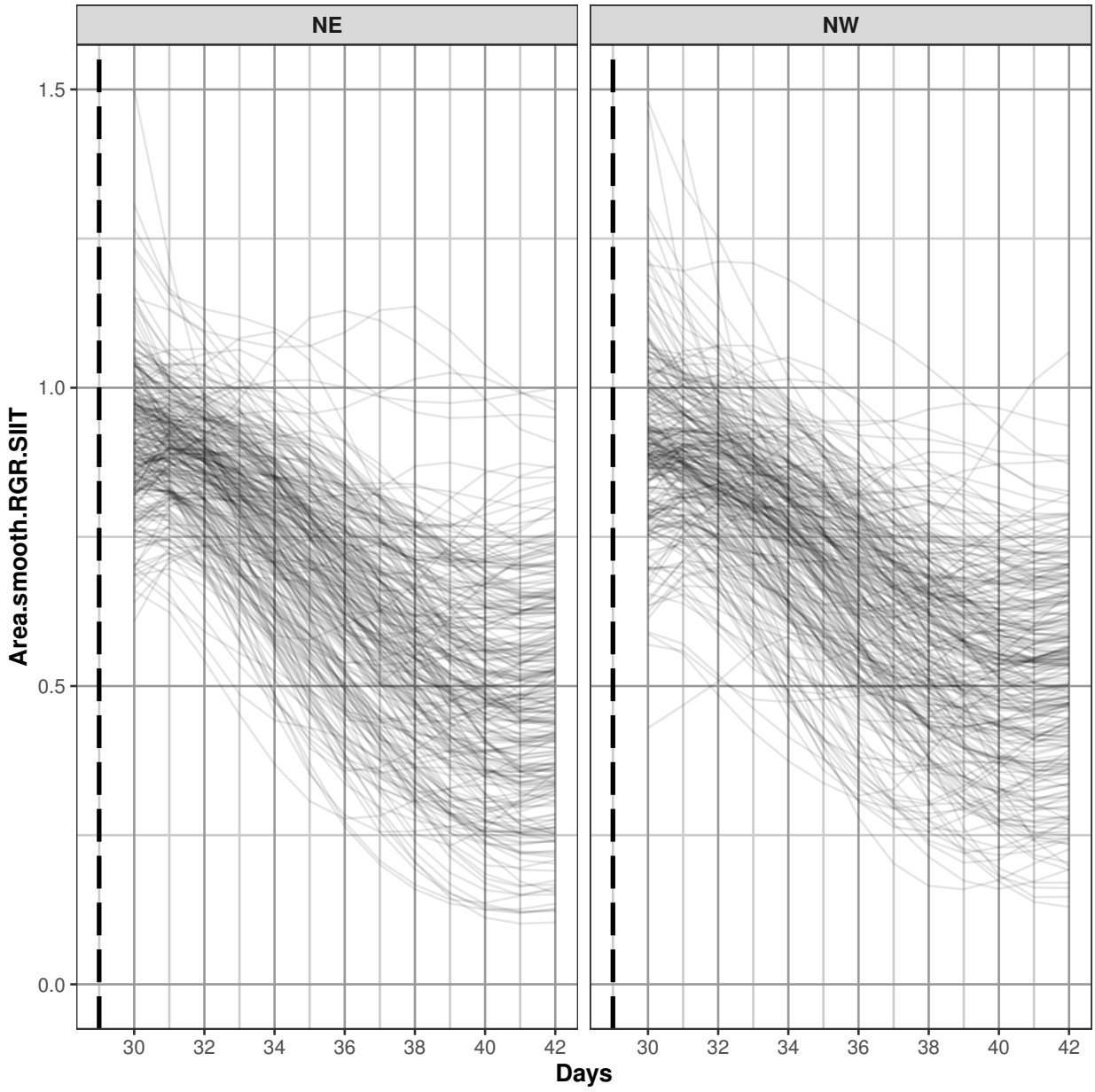
```
cols.retained <-  c("Snapshot.ID.Tag","Smarthouse","Lane","Position",
                    "Days","Snapshot.Time.Stamp", "Hour", "xDays",
                    "Zones","xZones","SHZones","ZLane","ZMainplots",
                    "xMainPosn", "Genotype.ID")
responses.GR <- c("Area.smooth.AGR","Area.smooth.AGR","Area.smooth.RGR")
suffices.results <- c("diff", "SIIT", "SIIT")
responses.SIIT <- unlist(Map(paste, responses.GR, suffices.results,sep="."))

longi.SIIT.dat <-
  twoLevelOpcreate(responses.GR, longi.dat, suffices.treatment=c("C","S"),
                   operations = c("-", "/", "/"), suffices.results = suffices.results,
                   columns.retained = cols.retained,
                   by = c("Smarthouse","Zones","ZMainplots","Days"))
longi.SIIT.dat <- with(longi.SIIT.dat,
                           longi.SIIT.dat[order(Smarthouse,Zones,ZMainplots,Days),])

# Plot SIIT profiles
k <- 2
nresp <- length(responses.SIIT)
limits <- with(longi.SIIT.dat, list(c(min(Area.smooth.AGR.diff, na.rm=TRUE),
                                      max(Area.smooth.AGR.diff, na.rm=TRUE)),
```

```
                                    c(0,3),
                                    c(0,1.5)))
#Plots
for (k in 1:nresp)
{
  plt <- plotLongitudinal(data = longi.SIIT.dat, x="xDays+35.42857143",
                          response = responses.SIIT[k],
                          y.title=responses.SIIT[k],
                          facet.x="Smarthouse", facet.y=".", printPlot=FALSE, )
  plt <- plt + geom_vline(xintercept=29, linetype="longdash", size=1) +
               scale_x_continuous(breaks=seq(28, 42, by=2)) +
               scale_y_continuous(limits=limits[[k]])
  print(plt)
}
```


NE
NW
0
−20
−40
−60
Area.smooth.AGR.diff
30
32
34
36
38
40
42
Days




```
## Warning: Removed 1 rows containing missing values (geom_path).
```



Calculate interval SIITs and check for large values for SIIT for Days 31to35

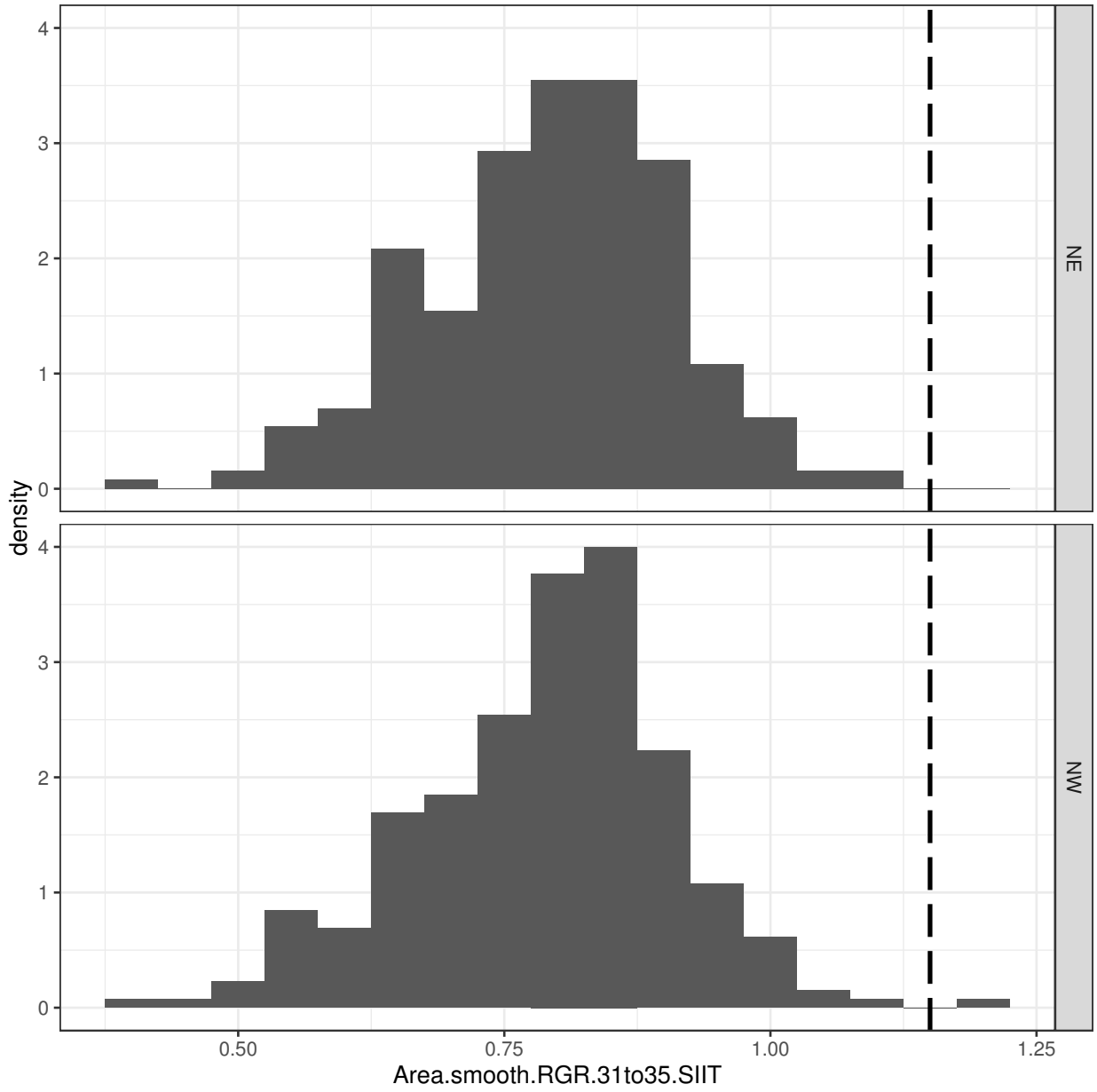
```
suffices <- list("31to35","35to38","31to38","38to42")
response <- "Area.smooth.RGR.31to35"
SIIT <- paste(response, "SIIT", sep=".")
responses.SIITinterval <- as.vector(outer("Area.smooth.RGR", suffices, paste, sep="."))

cart.SIIT.dat <- twoLevelOpcreate(responses.SIITinterval, cart.dat,
                                  suffices.treatment=c("C","S"),
                                  suffices.results="SIIT",
                                  columns.suffixed="Snapshot.ID.Tag")
tmp<-na.omit(cart.SIIT.dat)
print(summary(tmp[SIIT]))
```

```
##  Area.smooth.RGR.31to35.SIIT
##  Min.   :0.4077
##  1st Qu.:0.7160
##  Median :0.7999
##  Mean   :0.7908
##  3rd Qu.:0.8688
##  Max.   :1.1885
```

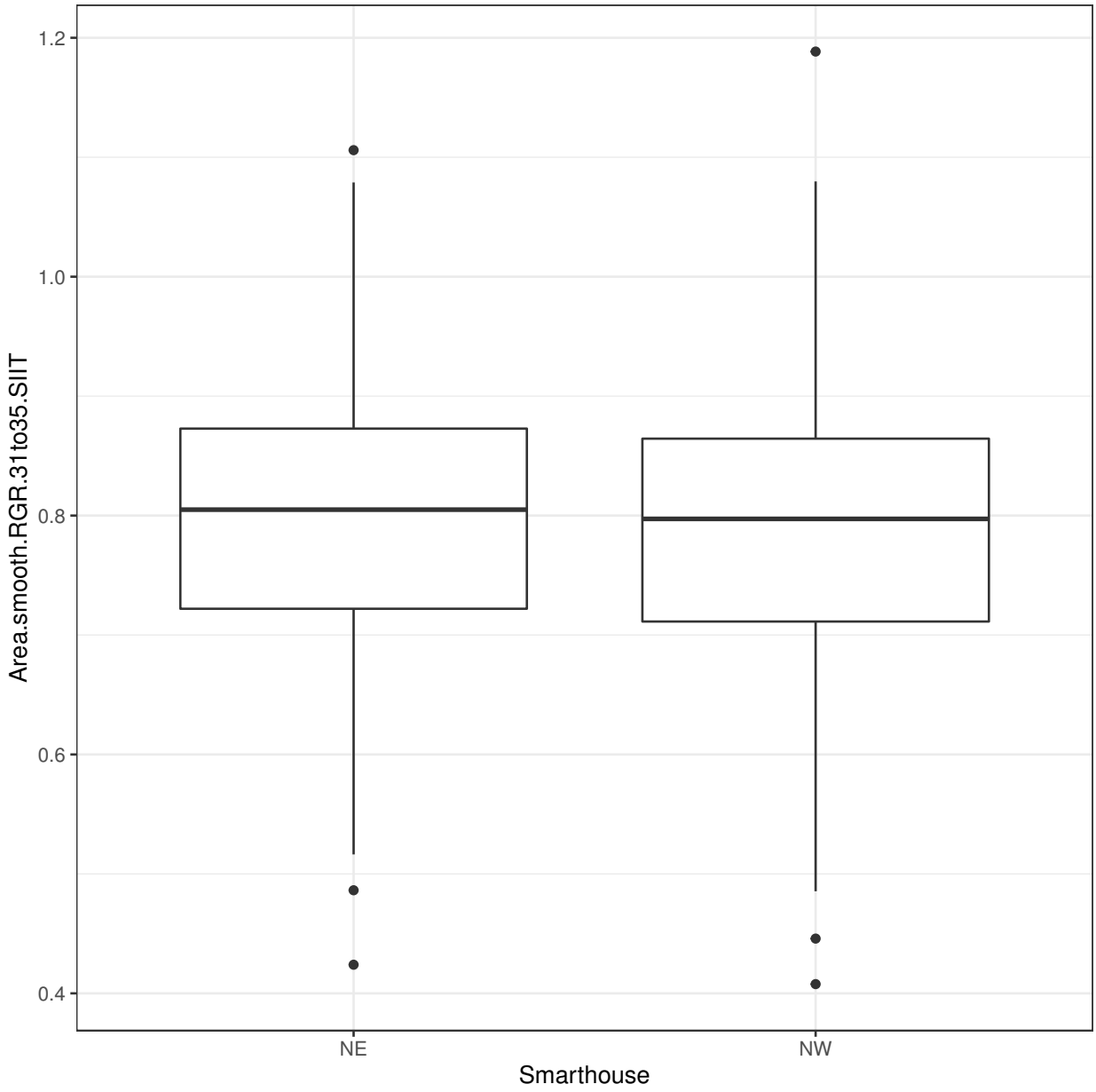
```
big.SIIT <- with(tmp, tmp[tmp[SIIT] > 1.15, c("Snapshot.ID.Tag.C","Genotype.ID",
                                              paste(response,"C",sep="."),
                                              paste(response,"S",sep="."), SIIT)])
big.SIIT <- big.SIIT[order(big.SIIT[SIIT]),]
print(big.SIIT)
```

```
##     Snapshot.ID.Tag.C Genotype.ID Area.smooth.RGR.31to35.C
## 325          046129-C      122090                0.1310631
##     Area.smooth.RGR.31to35.S Area.smooth.RGR.31to35.SIIT
## 325                0.1557642                    1.188467
```

```
plt <- ggplot(tmp, aes_string(SIIT)) +
          geom_histogram(aes(y = ..density..), binwidth=0.05) +
          geom_vline(xintercept=1.15, linetype="longdash", size=1) +
          theme_bw() + facet_grid(Smarthouse ~.)
print(plt)
```




```
plt <- ggplot(tmp, aes_string(x="Smarthouse", y=SIIT)) +
          geom_boxplot() + theme_bw()
print(plt)
```




```
remove(tmp)
```

Save image

```
save.image("Rice.RData")
```

References

Al-Tamimi, N, Brien, C.J., Oakey, H., Berger, B., Saade, S., Ho, Y. S., Schmockel, S. M., Tester, M. and Negrao, S. (2016) New salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping. *Nature Communications*, **7**, 13342.

Brien, C., Jewell, N., Garnett, T., Watts-Williams, S. J., & Berger, B. (2020). Smoothing and extraction of

traits in the growth analysis of noninvasive phenotypic data. *Plant Methods*, **16**, 36. http://dx.doi.org/10.1186/s13007-020-00577-6.