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

Chris Brien

12 December, 2020

This example is taken from Brien et al. (2020), who used it to illustrated the five steps of the method they describe for smoothing and extracting traits (SET). More details on the rationale for the steps used in this process are available in the Methods section of Brien et al. (2020).

Initialize

Set up characters for variable names and titles

```
responses <- c("Area", paste("Area", c("AGR", "RGR"), sep = "."))
responses.smooth <- c("Area.smooth", paste("Area.smooth", c("AGR", "RGR"), sep = "."))
responses.logis <- paste(responses.smooth, "Logistic", sep = ".")
resptitles <- c("PSA", "PSA AGR", "PSA RGR")
resptitles.smooth <- c("sPSA", "sPSA AGR", "sPSA RGR")
respunits <- c("(kpixels)", "(kpixels / day)", "( / day)")
y.titles <- c("PSA (kpixels)", "PSA AGR (kpixels / day)", "PSA RGR ( / day)")
names(y.titles) <- responses.smooth
ypred.titles <- paste0("Predicted s", y.titles)
names(ypred.titles) <- responses.smooth
pred.type <- c("Predicted", "Backtransformed predicted")
devn.titles <- c("PSA deviation (kpixels)", "PSA AGR deviation (kpixels / day)",
                 "PSA RGR deviation ( / day)")
names(devn.titles) <- responses.smooth
x.title <- "DAP"
# Specify time intervals of homogeneous growth dynamics
DAP.cart   <- c(18,22,27,33,39,43,51)
DAP.starts <- DAP.cart[-length(DAP.cart)]
DAP.ends   <- DAP.cart[-1]
DAP.mids <- (DAP.starts + DAP.ends)/2
#Functions to label the plot facets
labelAMF <- as_labeller(function(lev) paste(lev, "AMF"))
labelZn <- as_labeller(function(lev) paste("Zn:", lev, "mg/kg"))
vline.water <- list(geom_vline(xintercept=39, linetype="longdash",
                               alpha = 0.5, size=0.6))
x.axis <- list(scale_x_continuous(breaks = seq(17, 51, by = 2)),
               theme(axis.text.x = element_text(angle = 90),
                     panel.grid.minor.x = element_blank()))
vline.DAP.intvl <- list(geom_vline(xintercept=DAP.starts[-1], linetype="longdash",
                                   alpha = 0.5, size=0.75))
theme.profile <- list(vline.DAP.intvl,x.axis)
```

Step 1: Import, select and derive longitudinal data

In this step, the aim is to produce the data.frame `longit.dat` that contains the imaging variables, observed growth rates, covariates and factors. The growth rates are calculated from the observed data by differencing consecutive observations for a plant..

Load the pre-prepared data

```
data(tomato.dat)
```

Copy the data to preserve the original data.frame

```
longit.dat <- tomato.dat
```

Add continuous growth rates for raw data

```
longit.dat <- splitContGRdiff(longit.dat, responses = responses[1],
                              INDICES="Snapshot.ID.Tag", which.rates = c("AGR", "RGR"),
                              times.factor="DAP")
```

Step 2: Exploratory analysis

We begin by trying direct smoothing of the observed PSA with smoothing DF = 5. The growth rates are calculated from the smoothed data by difference, rather than from the spline derivatives. This matches the method used for the observed data.

Add smoothed PSA for logarithmic smoothing with DF = 6 to a temporary file

```
SET.dat <- longit.dat
SET.dat <- splitSplines(SET.dat, response = responses[1], x ="xDAP",
                        INDICES = "Snapshot.ID.Tag",
                        smoothing.method = "log", df = 6)
```

Add growth rates to SET.dat

```
SET.dat <- splitContGRdiff(SET.dat, responses = responses.smooth[1],
                           INDICES="Snapshot.ID.Tag", which.rates = c("AGR", "RGR"),
                           times.factor="DAP")
```

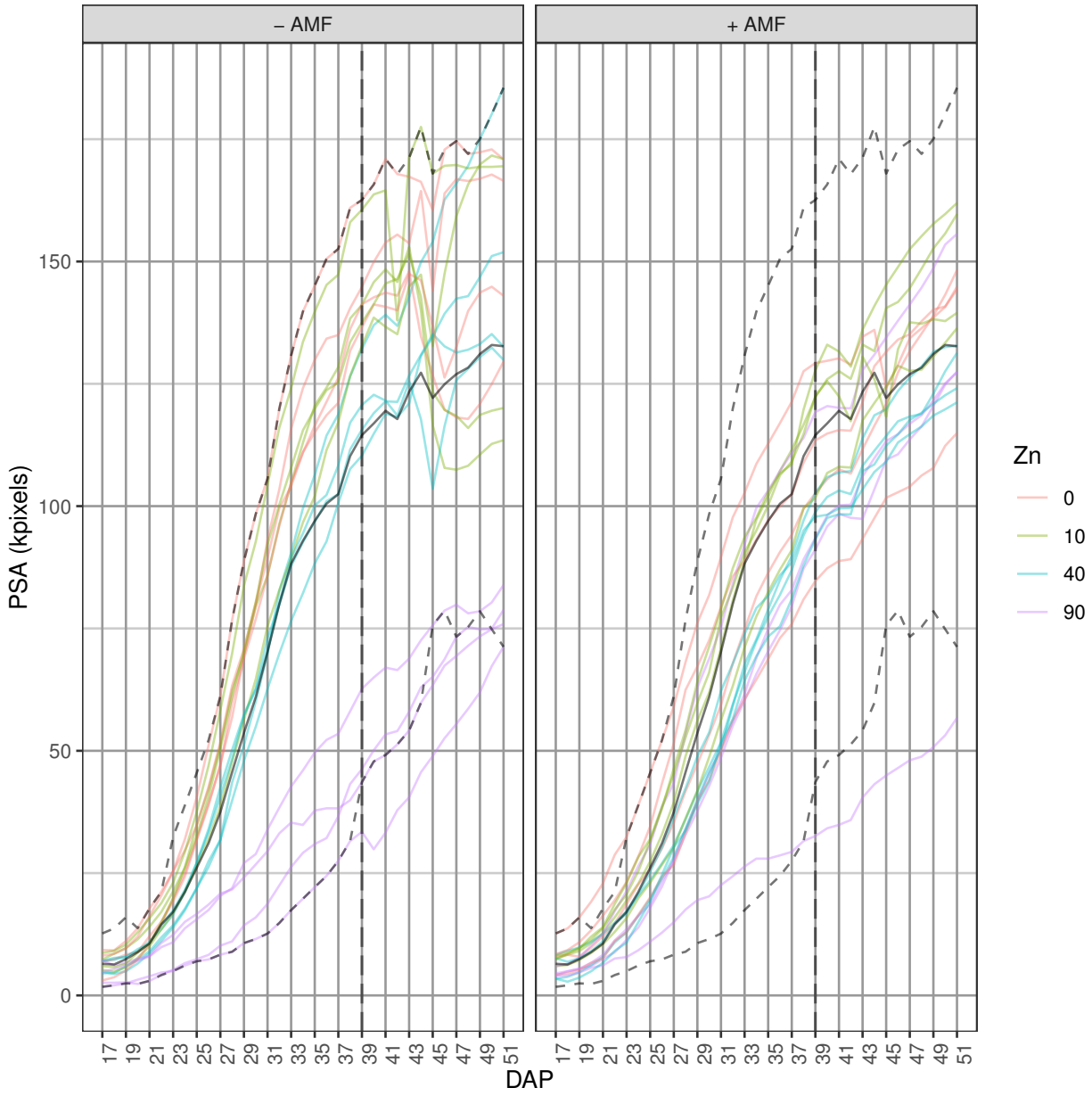
Plot the PSA traits

```
for (k in 1:length(responses))
{
  plotLongitudinal(SET.dat, x = "xDAP+34", response = responses[k],
                   xname = "xDAP", x.title = "DAP", y.title = paste(resptitles[k], respunits[k]),
                   facet.x = ".", facet.y = ".", alpha = 0.4,
                   colour.column = "Zn", addMediansWhiskers = TRUE,
                   ggplotFuncs = c(list(facet_grid(. ~ AMF,
                                                   labeller = labeller(AMF = labelAMF))),
                                   x.axis, vline.water))
  plotLongitudinal(SET.dat, x = "xDAP+34", response = responses.smooth[k],
```

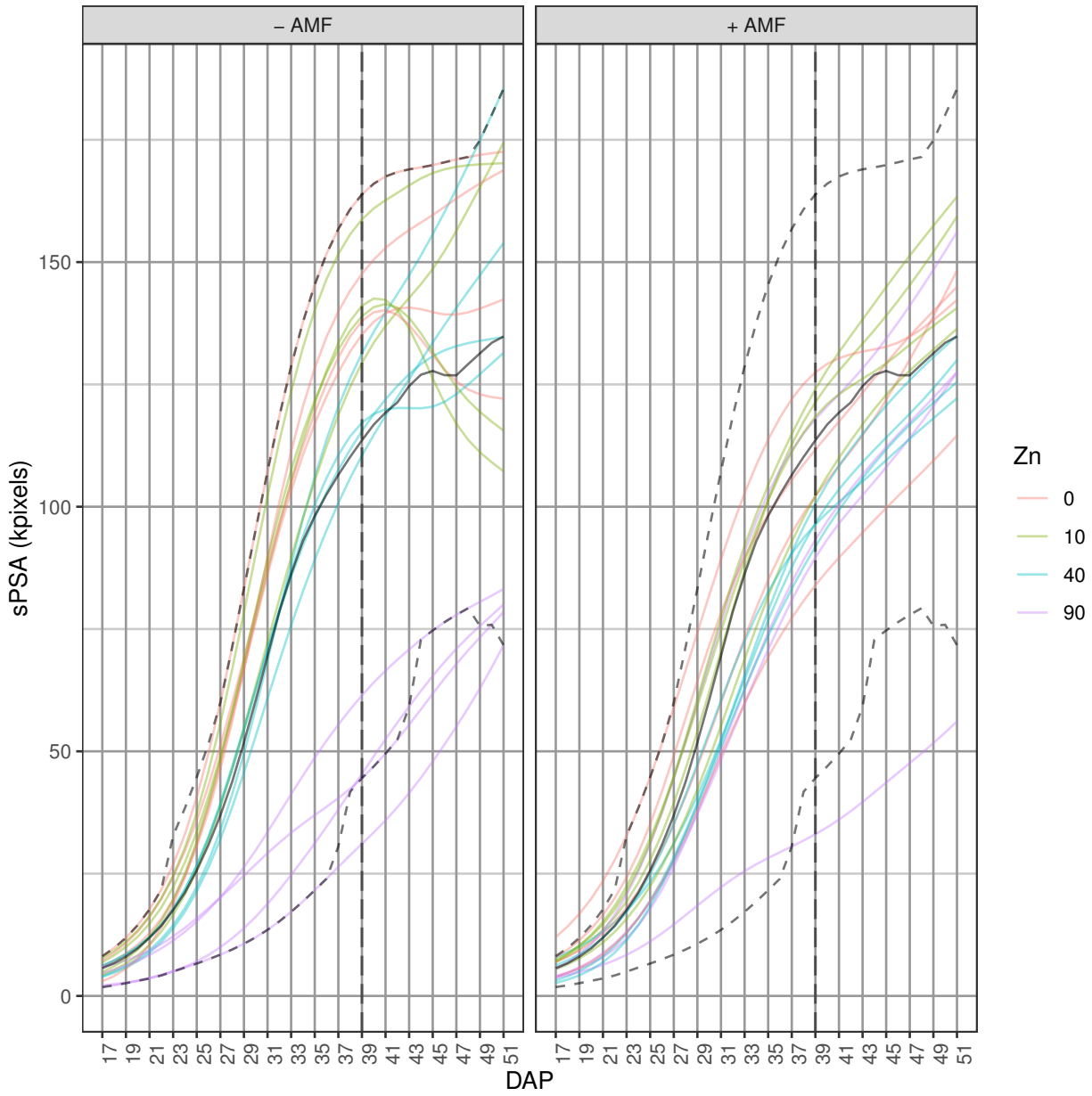
```
                    xname = "xDAP", x.title = "DAP",
                    y.title = paste(resptitles.smooth[k], respunits[k]),
                    facet.x = ".", facet.y = ".", alpha = 0.4,
                    colour.column = "Zn", addMediansWhiskers = TRUE,
                    ggplotFuncs = c(list(facet_grid(. ~ AMF,
                                                    labeller = labeller(AMF = labelAMF))),
                                    x.axis, vline.water))
}
```

```
## Warning in plotLongitudinal(SET.dat, x = "xDAP+34", response = responses[k], : x is xDAP+34 and xname
## Is xname the name of the column from which x is derived?
```

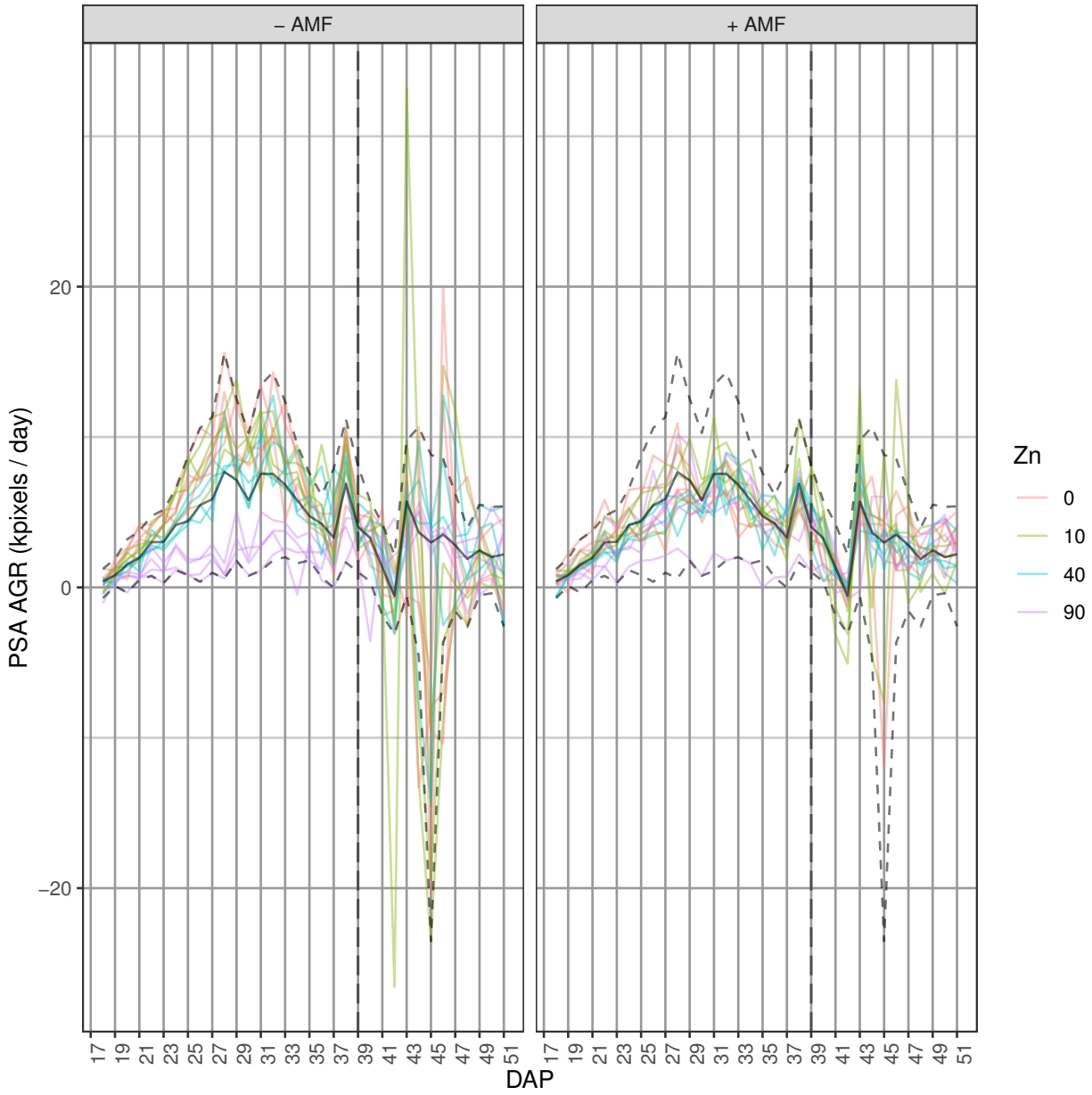
```
## Warning in plotLongitudinal(SET.dat, x = "xDAP+34", response = responses.smooth[k], : x is xDAP+34 an
## Is xname the name of the column from which x is derived?
```



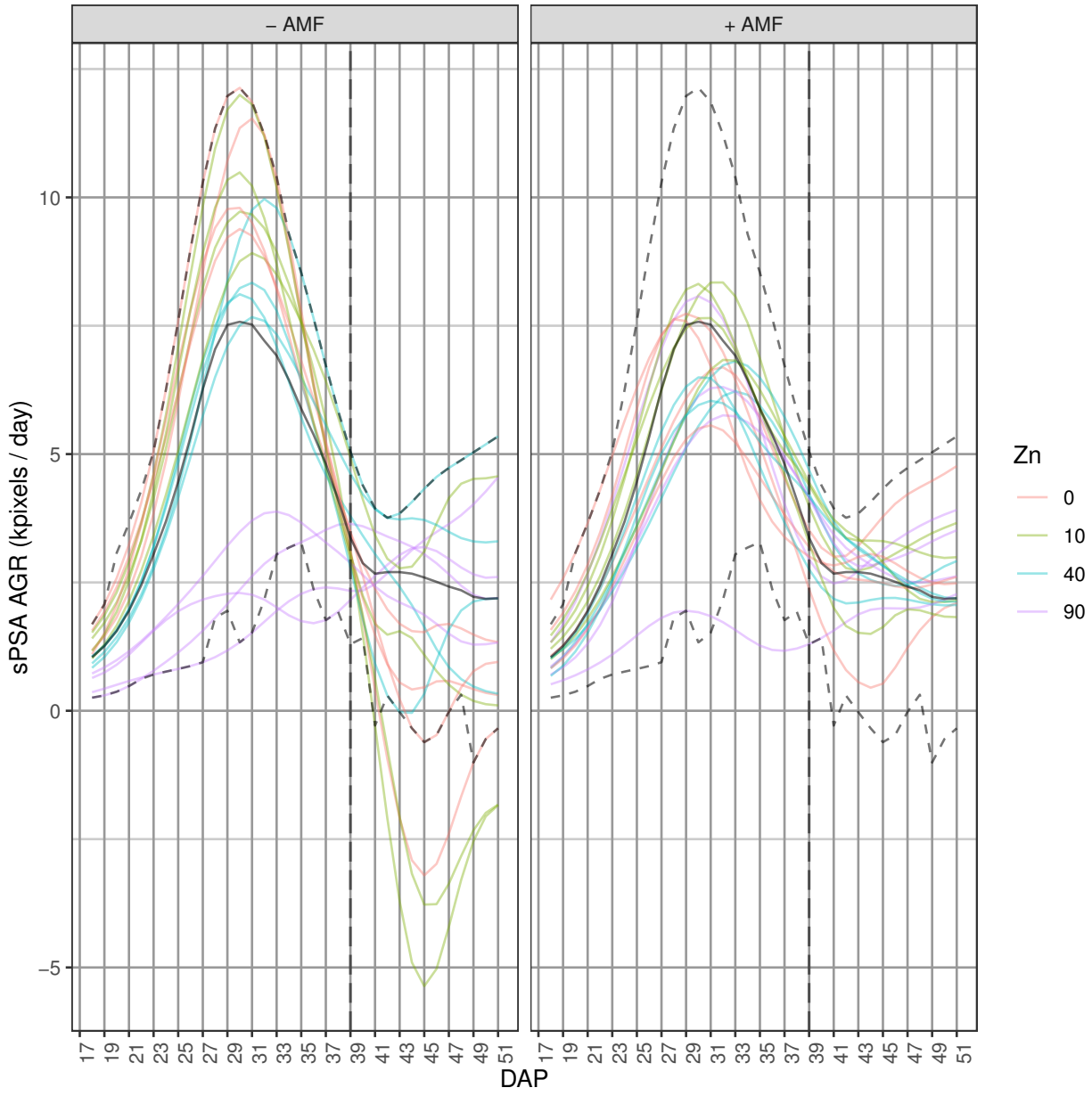
```
## Warning in plotLongitudinal(SET.dat, x = "xDAP+34", response = responses[k], : x is xDAP+34 and xname
## Is xname the name of the column from which x is derived?
```



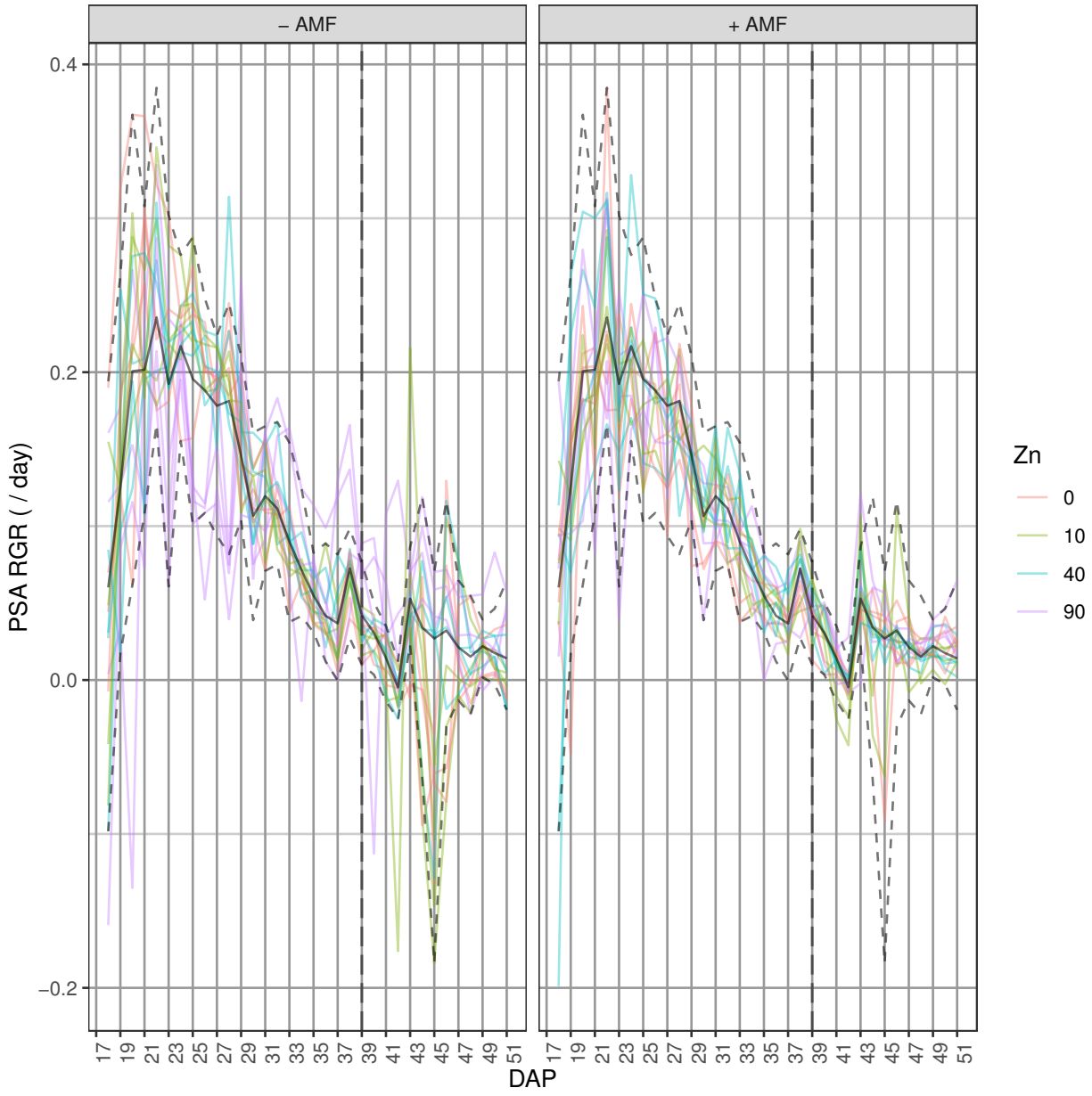

```
## Warning in plotLongitudinal(SET.dat, x = "xDAP+34", response = responses.smooth[k], : x is xDAP+34 a
## Is xname the name of the column from which x is derived?
```

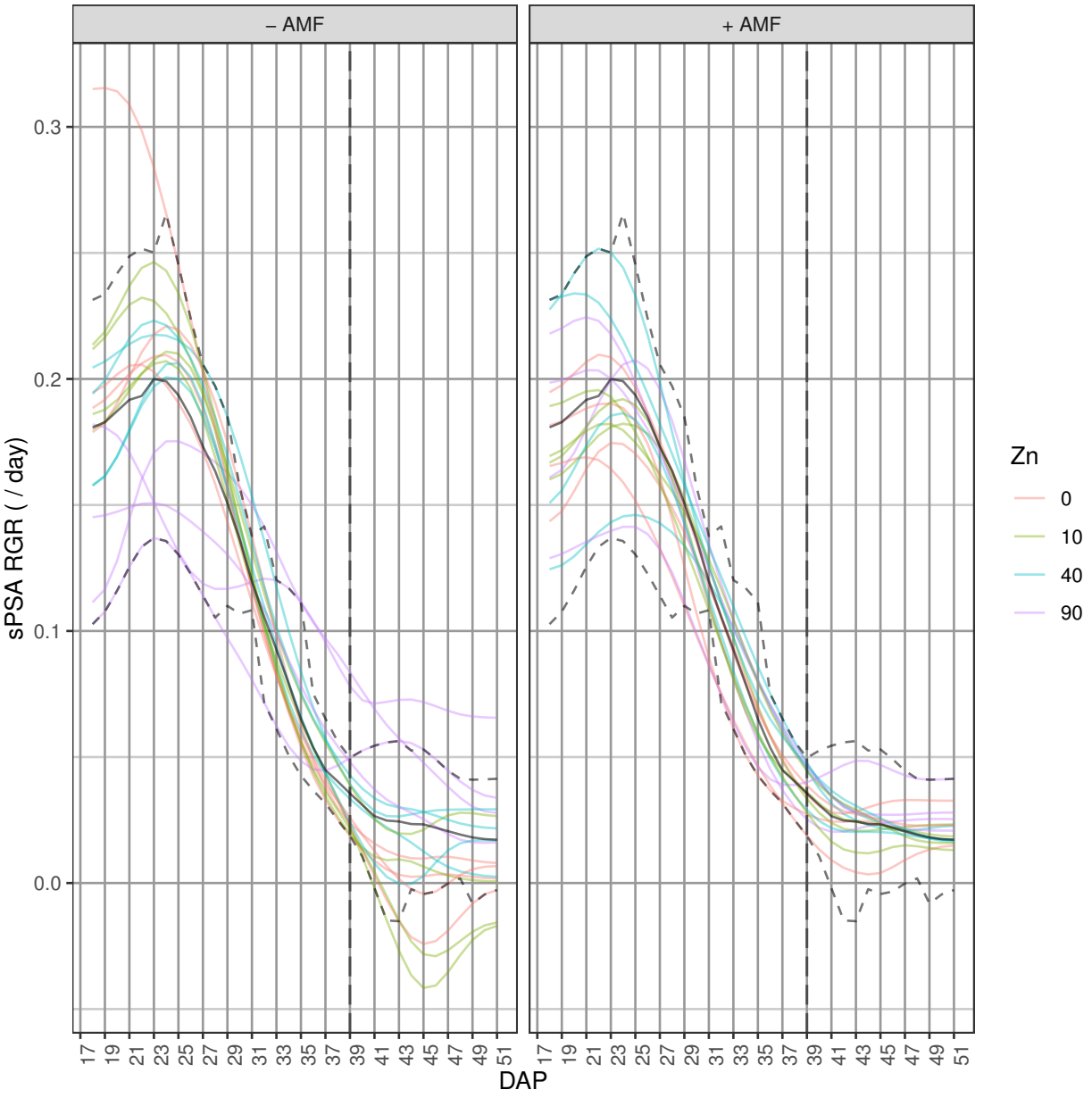



```
## Warning in plotLongitudinal(SET.dat, x = "xDAP+34", response = responses[k], : x is xDAP+34 and xname
## Is xname the name of the column from which x is derived?
```




```
## Warning in plotLongitudinal(SET.dat, x = "xDAP+34", response = responses.smooth[k], : x is xDAP+34 ar
## Is xname the name of the column from which x is derived?
```


– AMF
+ AMF
PSA RGR (/ day)
0.4
0.2
0.0
−0.2
17
19
21
23
25
27
29
31
33
35
37
39
41
43
45
47
49
51
DAP
Zn
0
10
40
90



Step 3: Choose smoothing DF and method

Fit three-parameter logistic curves

Organize non-missing data into a grouped object

```
logist.sub <- na.omit(longit.dat)
logist.grp <- groupedData(Area ~ xDAP | Snapshot.ID.Tag,
                          data = logist.sub)
```

Fit the logistics and obtain fitted values

```
logist.lis <- nlsList(SSlogis, logist.grp)
logist.sub$Area.smooth <- fitted(logist.lis)
```

Calculate the growth rates from the logistic fits

```
logist.sub <- splitContGRdiff(logist.sub, responses = responses.smooth[1],
                              INDICES="Snapshot.ID.Tag", which.rates = c("AGR", "RGR"),
                              times.factor="DAP")
names(logist.sub)[match(responses.smooth, names(logist.sub))] <- responses.logis
```

Probe the smoothing methods and DF

```
smooth.dat <- probeSmoothing(data = longit.dat, response = "Area",
                             xname = "xDAP", times.factor="DAP",
                             facet.x = ".", facet.y = ".",
                             smoothing.methods = c("dir", "log"),
                             df = c(4:6,12), x="xDAP", get.rates = TRUE,
                             which.plots = "none", deviations.plots = "none")
```

```
## Warning in log(PGR(x, time.diffs, lag = lag)): NaNs produced

## Warning in log(PGR(x, time.diffs, lag = lag)): NaNs produced

## Warning in log(PGR(x, time.diffs, lag = lag)): NaNs produced
```

```
SET.dat <- merge(longit.dat, smooth.dat)
SET.dat <- merge(SET.dat, logist.sub, all.x = TRUE)
```

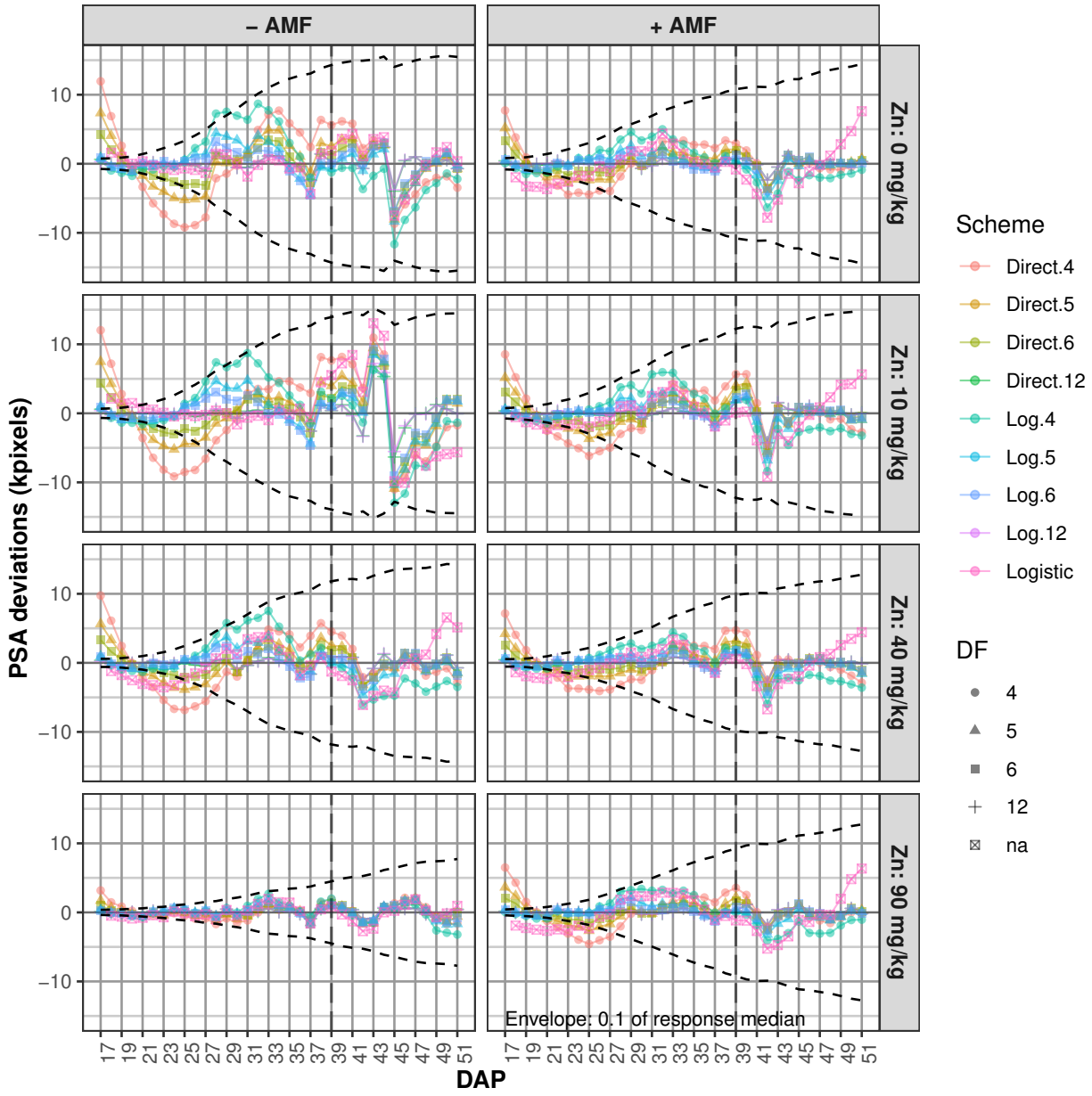
Plot the median deviations plots

```
propn.types = c(0.1, 0.5, 0.75)
devn.scales <- list()
devn.scales$Area.smooth     <- scale_y_continuous(#limits = c(-15,15),
  breaks = seq(-10,10,by=10))
devn.scales$Area.smooth.AGR <- scale_y_continuous(limits = c(-10,10),
                                                  breaks = seq(-10,10,by=5))
devn.scales$Area.smooth.RGR <- scale_y_continuous(limits = c(-0.75,0.25),
                                                  breaks = round(seq(-0.75,0.25,by=0.25), 2))
for (k in 1:length(responses))
  plotMedianDeviations(data = SET.dat,
                       response =responses[k],
                       response.smoothed = responses.smooth[k],
                       extra.smooths = "Logistic",
                       x = "xDAP+34", xname = "xDAP", x.title = x.title,
                       smoothing.methods = c("dir", "log"), df = c(4:6,12) ,
                       y.titles = paste(resptitles[k], "deviations", respunits[k]),
                       facet.x = "AMF", facet.y = "Zn",
                       trait.types = "response",
                       propn.types = propn.types[k],
                       labeller = labeller(Zn = labelZn,
                                           AMF = labelAMF),
```

```
                ggplotFuncsMedDevn = c(devn.scales[k], vline.water, x.axis))
```

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

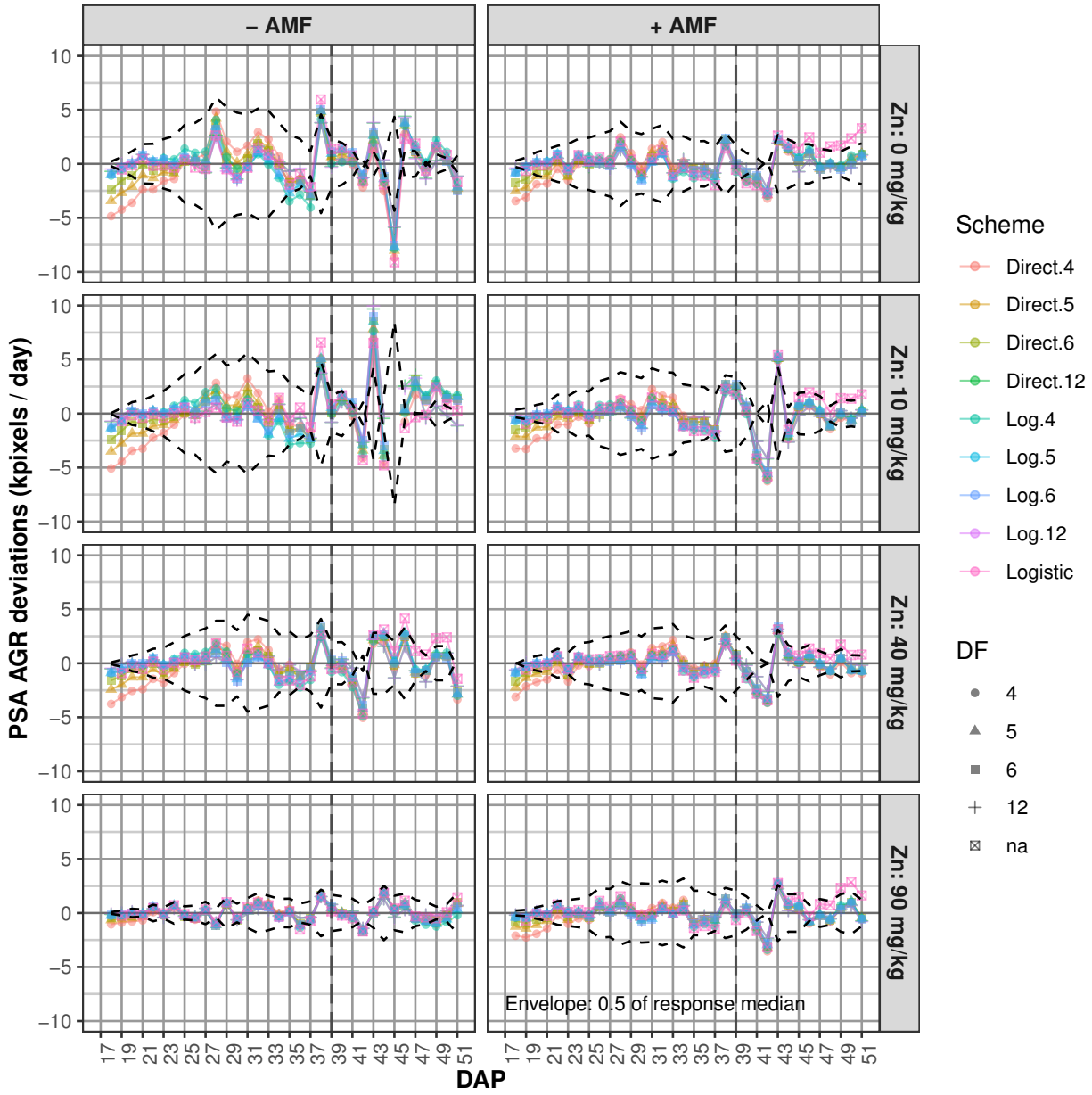
```
## Warning: Removed 8 rows containing missing values (geom_point).
```



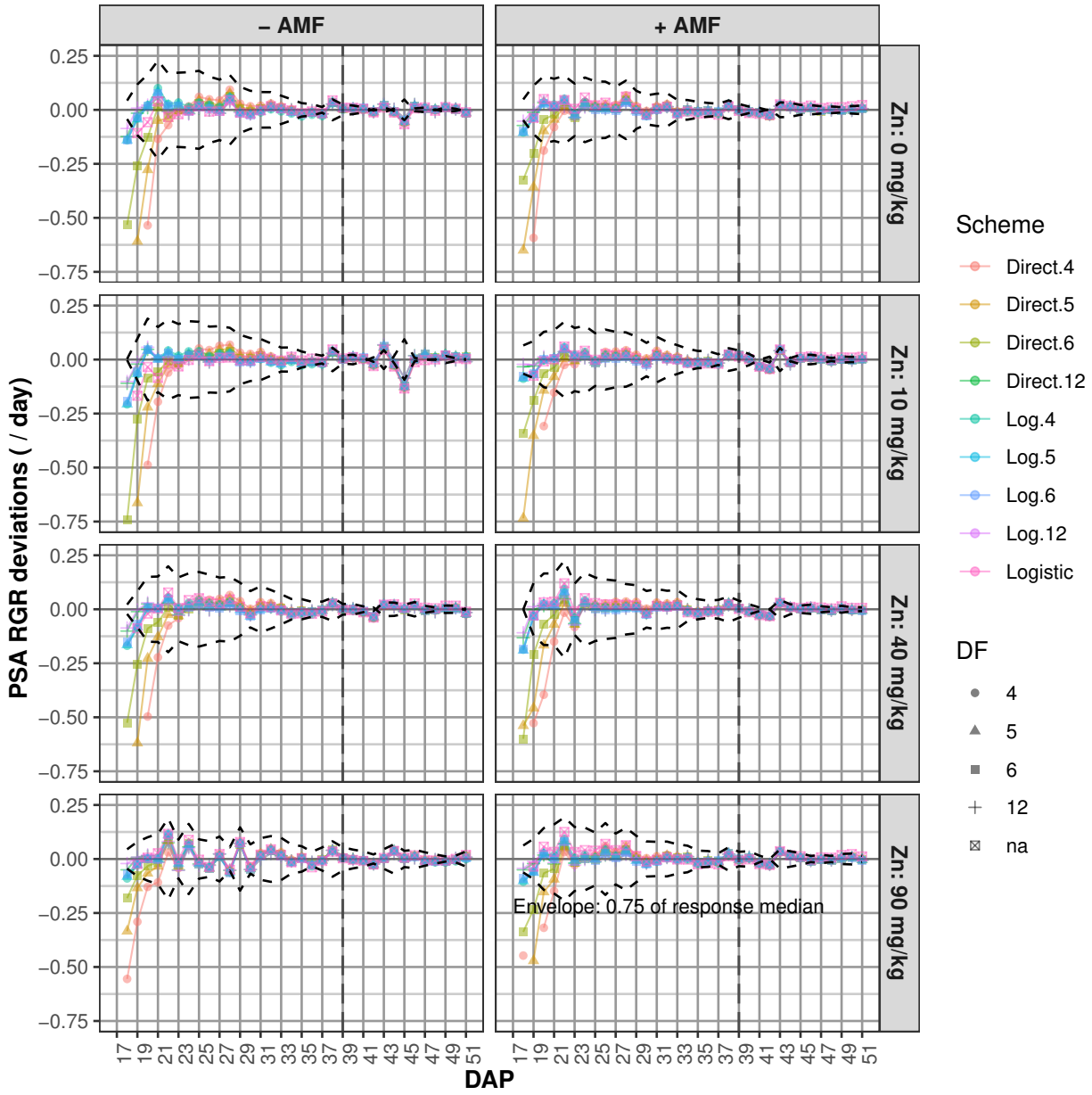

```
## Warning: Removed 10 row(s) containing missing values (geom_path).
```

```
## Warning: Removed 89 rows containing missing values (geom_point).
```

```
## Warning: Removed 2 row(s) containing missing values (geom_path).
```




```
## Warning: Removed 13 row(s) containing missing values (geom_path).
## Warning: Removed 95 rows containing missing values (geom_point).
## Warning: Removed 2 row(s) containing missing values (geom_path).
```



Plot the profile plots comparing direct and log smoothing for DF = 6

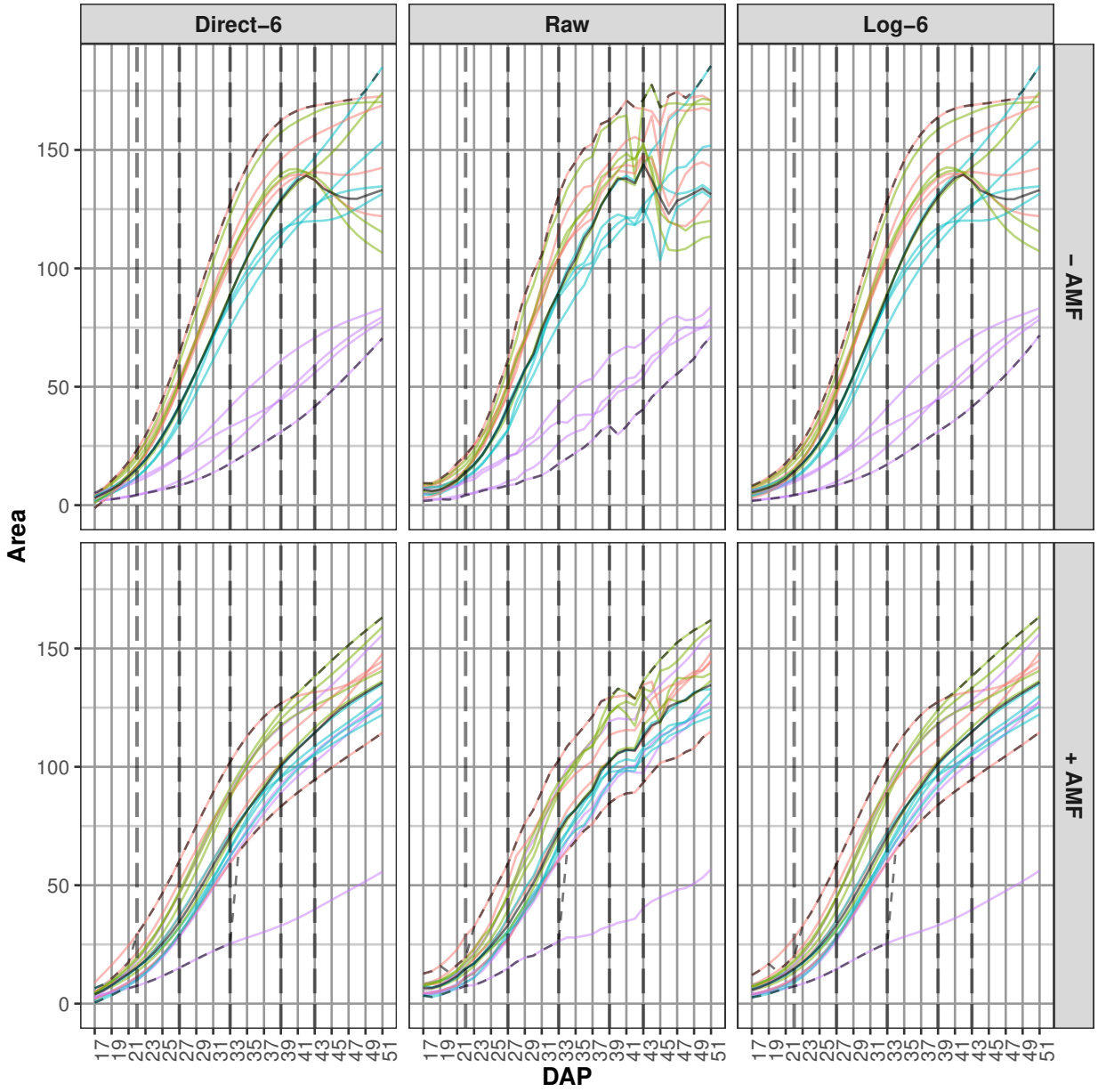
```
probeSmoothing(data = SET.dat, response = responses[1],
               x = "xDAP+34", xname = "xDAP", x.title = x.title,
               times.factor="DAP",
               facet.x = ".", facet.y = "AMF",
               smoothing.methods = c("dir", "log"),
               df = 6, get.rates = TRUE,
               colour.column = "Zn", alpha = 0.5,
               which.plots = "methods+rawcompare", addMediansWhiskers = TRUE,
               deviations.plots = "none",
               labeller = labeller(Zn = labelZn,
                                   AMF = labelAMF),
```

```
                ggplotFuncs = theme.profile)
```

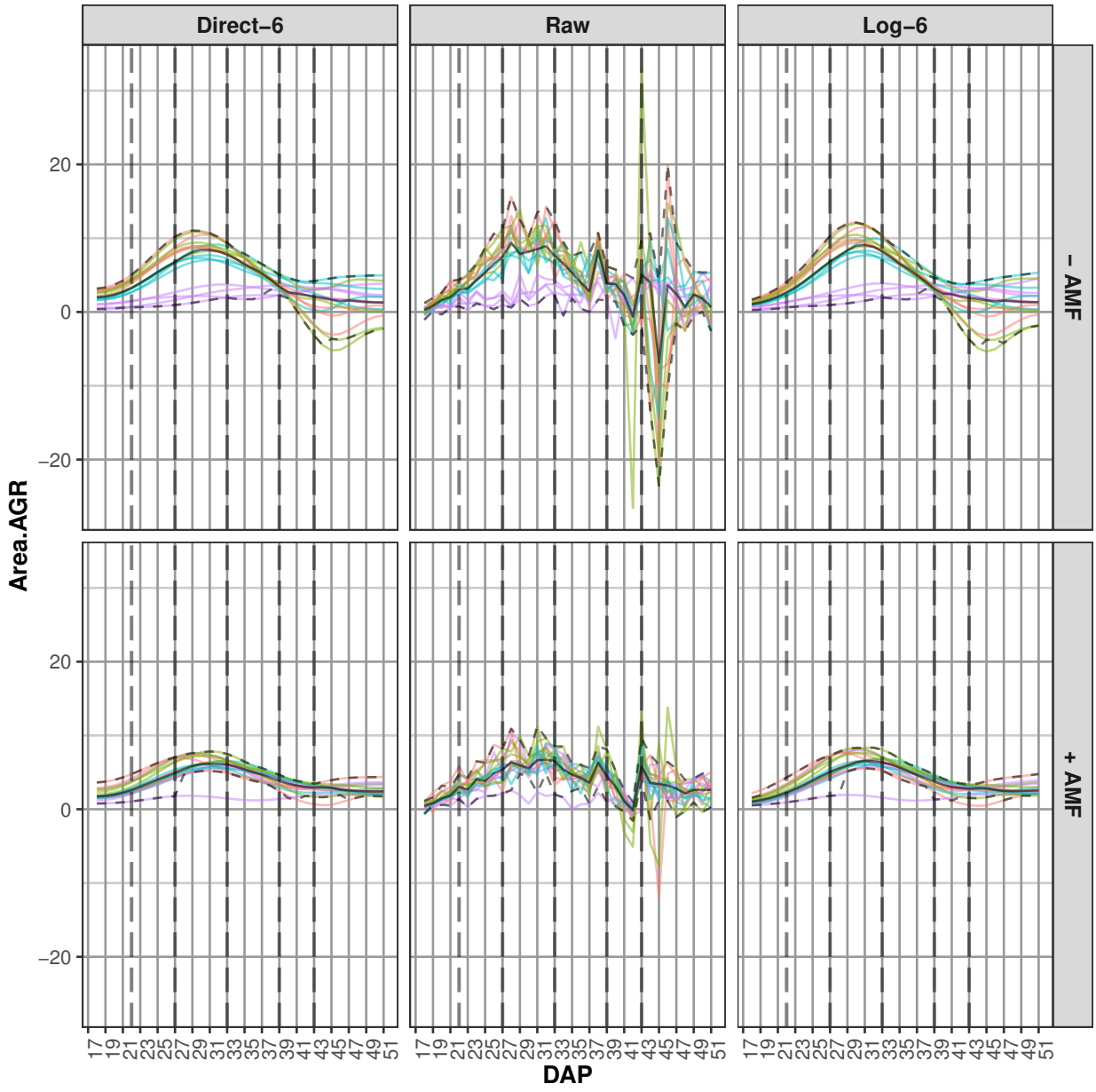
```
## Warning in log(PGR(x, time.diffs, lag = lag)): NaNs produced
```

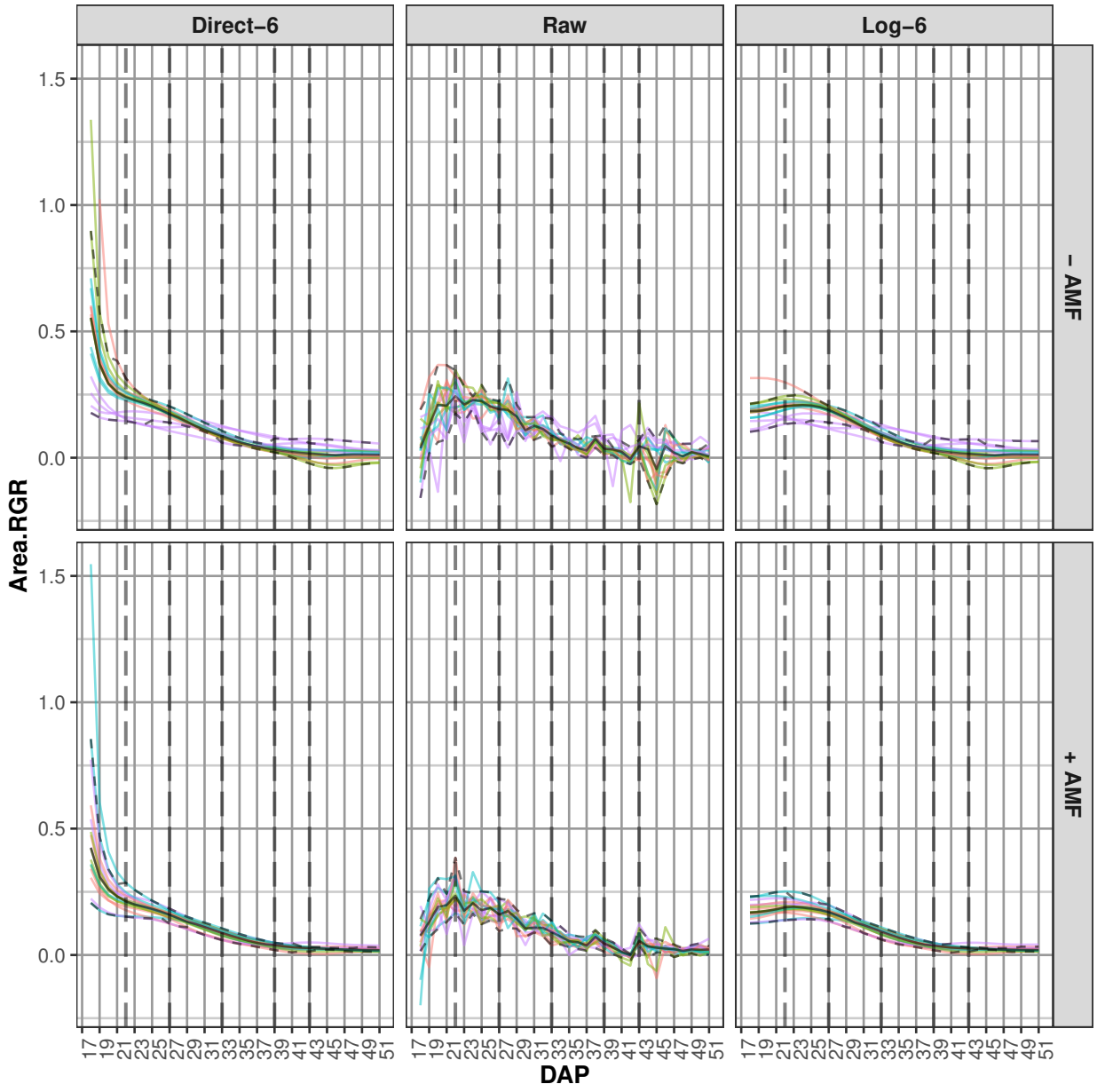
```
## Warning in plotLongitudinal(data = tmp.sm, x = x, xname = xname, response = response, : x is xDAP+34
## Is xname the name of the column from which x is derived?
```

```
## Warning in plotLongitudinal(data = tmp.sm, x = x, xname = xname, response = response, : x is xDAP+34
## Is xname the name of the column from which x is derived?
```




```
## Warning in plotLongitudinal(data = tmp.sm, x = x, xname = xname, response = response, : x is xDAP+34
## Is xname the name of the column from which x is derived?
```


Direct−6
Raw
Log−6
− AMF
+ AMF
Area.AGR
20
0
−20
17
19
21
23
25
27
29
31
33
35
37
39
41
43
45
47
49
51
DAP



Step 4: Identify potential outliers and remove if justified

A plant was identified as slow growing. Even though its pot had been inoculated with AMF, it had low AMF root colonization and a random mutated shoot phenotype, which could explain why its behaviour was consistent with a plant that was not inoculated with AMF. We omit the it from further analysis.

Omit responses for the outlier plant

```
omit <- with(longit.dat, Zn==90 & AMF=="+" & Block ==4)
responses.all <- names(longit.dat)[match("Weight.After", names(longit.dat)):length(longit.dat)]
longit.dat[omit, responses.all] <- NA
```

Step 3 (cont'd): Choose smoothing DF and method

Add smoothed PSA for log-smoothing with DF = 6

```
longit.dat <- splitSplines(longit.dat, response = responses[1], x ="xDAP",
                           INDICES = "Snapshot.ID.Tag",
                           smoothing.method = "log", df = 6)
```

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

Add growth rates to longit.dat

```
longit.dat <- splitContGRdiff(longit.dat, responses = responses.smooth[1],
                              INDICES="Snapshot.ID.Tag", which.rates = c("AGR", "RGR"),
                              times.factor="DAP")
```

Step 5: Per-cart trait formulation and extraction

In this step, traits that hace a single-value for each sharp are created.

The time intervals of homogeneous growth dynamics (set in global.r)

```
(suffices   <- paste(DAP.starts, DAP.ends, sep="to"))
```

```
## [1] "18to22" "22to27" "27to33" "33to39" "39to43" "43to51"
```

Commence cart.dat

```
nidcols <- match("Weight.After", names(longit.dat))-1
idcols.cart <- names(longit.dat)[1:nidcols]
cart.dat <- longit.dat[longit.dat$DAP == DAP.cart[1], idcols.cart]
cart.dat <- cart.dat[do.call(order, cart.dat), ]
```

Populate cart.dat at prescribed time-points

```
for (day in DAP.cart)
  cart.dat <- cbind(cart.dat,
                    getTimesSubset(responses.smooth, data = longit.dat,
                                   times.factor = "DAP", which.times = day,
                                   suffix = as.character(day)))
```

Populate cart.dat and cart.allt.intervals at prescribed intervals

```
for (r in responses.smooth[1])
{
  for (k in 1:length(suffices))
  {
    cart.dat <- merge(cart.dat,
                      intervalGRdiff(r,
                                     which.rates = c("AGR","RGR"),
                                     times.factor = "DAP",
```

```
                                    start.times = DAP.starts[k],
                                    end.times = DAP.ends[k],
                                    suffix.interval = suffices[k],
                                    data = longit.dat),
                        by = "Snapshot.ID.Tag")

  }
}
```

Finalise

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

```
##   Snapshot.ID.Tag DAP Lane Position xDAP DAP.diffs xPosn Block Cart AMF Zn
## 1          061472  18    6        5  -16         1  -8.5     1    1   -  0
## 2          061473  18    6        6  -16         1  -7.5     1    2   + 10
## 3          061474  18    6        7  -16         1  -6.5     1    3   - 90
## 4          061475  18    6        8  -16         1  -5.5     1    4   + 40
## 5          061476  18    6        9  -16         1  -4.5     1    5   + 90
## 6          061477  18    6       10  -16         1  -3.5     1    6   - 40
##   Treatments Area.smooth.18 Area.smooth.AGR.18 Area.smooth.RGR.18
## 1        -,0       9.807324          1.6845547          0.1884583
## 2       +,10       8.162253          1.2076831          0.1601212
## 3       -,90       2.420749          0.2552224          0.1114134
## 4       +,40       8.903409          1.0421415          0.1244864
## 5       +,90       4.741824          0.7045944          0.1608632
## 6       -,40       4.983090          0.9214957          0.2044746
##   Area.smooth.22 Area.smooth.AGR.22 Area.smooth.RGR.22 Area.smooth.27
## 1      21.757197          4.0535127          0.2061718      59.936608
## 2      16.096746          2.6186640          0.1775523      39.409717
## 3       4.191419          0.6203235          0.1601669       9.954592
## 4      15.116144          1.9650536          0.1392586      31.134433
## 5       9.600854          1.6661982          0.1906121      26.442502
## 6      11.630394          2.2636744          0.2164589      33.821095
##   Area.smooth.AGR.27 Area.smooth.RGR.27 Area.smooth.33 Area.smooth.AGR.33
## 1          10.292807          0.1884140      128.71939          10.245261
## 2           6.243039          0.1724667       86.91303           8.071984
## 3           1.560483          0.1705038       24.72807           3.047349
## 4           4.142143          0.1427631       64.69042           6.223293
## 5           4.704429          0.1959074       62.49983           6.215004
## 6           6.288936          0.2057300       88.28055           9.794637
##   Area.smooth.RGR.33 Area.smooth.39 Area.smooth.AGR.39 Area.smooth.RGR.39
## 1         0.08294015      163.83401           3.038382         0.01871961
## 2         0.09747422      121.36293           3.981731         0.03335872
## 3         0.13151559       45.37888           3.643014         0.08368594
## 4         0.10114846       96.64198           4.233163         0.04479083
## 5         0.10473886       93.25406           4.156842         0.04559948
## 6         0.11760066      131.36934           5.048666         0.03918904
##   Area.smooth.43 Area.smooth.AGR.43 Area.smooth.RGR.43 Area.smooth.51
## 1      168.95836          0.5494274         0.00325715      172.57053
## 2      133.31548          2.7296263         0.02068746      159.31254
## 3       59.16752          3.3001120         0.05739157       80.01691
```

```
## 4      109.11412          2.6781128         0.02485037      129.95768
## 5      106.62596          2.9949757         0.02849065      127.50507
## 6      147.30562          3.8417482         0.02642624      185.43136
##   Area.smooth.AGR.51 Area.smooth.RGR.51 Area.smooth.AGR.18to22
## 1          0.3070212         0.00178069              2.9874681
## 2          3.6586227         0.02323287              1.9836231
## 3          2.1967173         0.02783704              0.4426675
## 4          2.9164823         0.02269743              1.5531839
## 5          2.6213027         0.02077269              1.2147575
## 6          5.3449009         0.02924772              1.6618259
##   Area.smooth.RGR.18to22 Area.smooth.AGR.22to27 Area.smooth.RGR.22to27
## 1              0.1992038               7.635882              0.2026686
## 2              0.1697742               4.662594              0.1790791
## 3              0.1372406               1.152635              0.1729989
## 4              0.1323323               3.203658              0.1445102
## 5              0.1763576               3.368330              0.2026241
## 6              0.2118929               4.438140              0.2134926
##   Area.smooth.AGR.27to33 Area.smooth.RGR.27to33 Area.smooth.AGR.33to39
## 1              11.463797              0.1273912               5.852438
## 2               7.917219              0.1318159               5.741651
## 3               2.462247              0.1516509               3.441801
## 4               5.592665              0.1218831               5.325260
## 5               6.009554              0.1433652               5.125705
## 6               9.076575              0.1599058               7.181466
##   Area.smooth.RGR.33to39 Area.smooth.AGR.39to43 Area.smooth.RGR.39to43
## 1             0.04020318               1.281087            0.007699625
## 2             0.05564626               2.988136            0.023483206
## 3             0.10118461               3.447160            0.066331488
## 4             0.06690002               3.118033            0.030345249
## 5             0.06669396               3.342975            0.033499857
## 6             0.06624883               3.984070            0.028624182
##   Area.smooth.AGR.43to51 Area.smooth.RGR.43to51
## 1              0.4515206            0.002644212
## 2              3.2496329            0.022268701
## 3              2.6061736            0.037733154
## 4              2.6054456            0.021851825
## 5              2.6098887            0.022353640
## 6              4.7657177            0.028771915
```

Save the cart.data and the workspace

```
save(cart.dat, file="../data/cart.dat.rda")
load(file="../data/cart.dat.rda")
save.image("Tomato.RData")
```

Reference

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.