

Todo list

| | |
|-------------------------------|----|
| Describe experiment | 24 |
|-------------------------------|----|

EDUARD SZÖCS

QUANTITATIVE ECOTOXICOLOGY

WITH R!

This document was created using \LaTeX , knitr and the tufte book class.

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1

Introduction

```
require(devtools)  
install_github("qetx", "EDiLD")
```

```
require(qetx)
```


2

The Measurement Process

2.1 Winsorized Mean and Standard Deviation

Example data

The following sulfate concentrations (mg/L) were measured during a routine water quality survey of the Savannah River (South Carolina). The data is available in the `qetx` package ¹:

```
data(so4)
```

```
so4
## [1] 1.3 2.3 2.6 3.3 3.5 3.5 3.6 4.0 4.1 4.5 5.2 5.6
## [13] 5.7 6.1 6.2 6.5 6.9 7.1 7.7 7.9 9.9

length(so4)

## [1] 21

mean(so4)

## [1] 5.119

sd(so4)

## [1] 2.137
```

So there are 21 measurements with a mean of 5.12 mg/L and a standard deviation of 2.14 mg/L.

Winsorization

Suppose we have a detection limit of 2.5 mg/L and want to winsorize values below LOD, i.e. replace the two lowest values by 2.6 mg/L and the two highest values by 7.7 mg/L.

¹ Note that in this case you do not have to assign the data to a name.

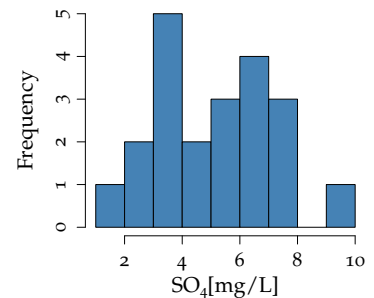


Figure 2.1: A histogram of the `so4` data.

Happily there is function in the `qetx`-package to do this for us: `winsor()`. This function takes a vector of values and a second argument specifying how many values should be winsorized (either by giving a LOD-value or the number of values on each side) ².

```
so4_w <- winsor(so4, lod = 2.5)
so4_w

## [1] 2.6 2.6 2.6 3.3 3.5 3.5 3.6 4.0 4.1 4.5 5.2 5.6
## [13] 5.7 6.1 6.2 6.9 6.5 7.1 7.7 7.7 7.7
## attr("width")
## [1] 2
```

² Look at the source of this function - type the function name into the console - to see which computations are done.

This give the expected results, moreover we see that on each end two observations where modified ³.

```
mean(so4_w)

## [1] 5.081

sd(so4_w)

## [1] 1.792

sd_winsor(so4_w)

## [1] 2.24
```

³ stored within the attribute 'width' of the resulting vector. **TODO: verbatim within sidenote.**

The Winsorized mean (\bar{x}_w) now is 5.08 mg/L, the standard deviation of the modified data set (s) is 1.79 mg/L and the Winsorized standard deviation (s_w) 2.24 mg/L.

2.2 Probability Plotting

3

Bioaccumulation

4

Tests for Detection of Chronic Lethal and Sub-lethal Stress

5

Lethal and Other Quantal Responses to Stress

5.1 Fitting dose-response models

Example data

Newman and Aplin (1992) exposed mosquitofish *Gambusia holbrooki* to a series of NaCl concentrations for 96h. The data is available from the `qetx` package.

```
data(salt)
str(salt)

## 'data.frame': 6 obs. of 3 variables:
## $ dead : int 16 22 40 69 78 77
## $ total: int 76 79 77 76 78 77
## $ conc : num 10.3 10.8 11.6 13.2 15.8 20.1
```

There are three columns:

dead Number of fish died.

total Total number of fish exposed.

conc NaCl concentration (g/L).

First we calculate the proportion of died fish and save it as a new column in our data.frame:

```
salt$prop <- salt$dead/salt$total
```

As always we first take a look at the data, to produce Fig. 5.2:

```
plot(prop ~ conc, data = salt, log = 'x', pch = 16,
     xlab = "NaCl-Concentration", ylab = "Proportion dead")
```

Introduction

1



Figure 5.1: LD₅₀. Source: <http://xkcd.com/1260/>

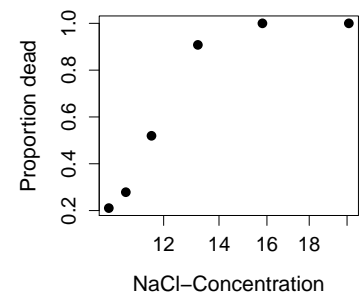


Figure 5.2: Proportion of fish died at different NaCl concentrations. The x axis is on a log scale.

¹ Ritz, 2010

6

Population and Metapopulation Effects

7

Community Effects

7.1 Species Richness

Example data

Ten species were sampled at eight sites around an outfall and the number of individuals per species noted. The data is available from the `qetx` package has a usual species x sites format and the row-names give the distance to the outfall¹:

```
data(abu)
abu
```

| ## | Sp1 | Sp2 | Sp3 | Sp4 | Sp5 | Sp6 | Sp7 | Sp8 | Sp9 | Sp10 |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| ## -1 | 12 | 11 | 10 | 8 | 8 | 5 | 2 | 2 | 1 | 0 |
| ## -0.5 | 12 | 12 | 10 | 8 | 8 | 4 | 3 | 2 | 2 | 1 |
| ## 0 | 58 | 21 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| ## 0.5 | 18 | 16 | 15 | 5 | 4 | 0 | 0 | 0 | 0 | 0 |
| ## 1 | 11 | 11 | 11 | 8 | 7 | 3 | 2 | 1 | 1 | 0 |
| ## 1.7 | 8 | 12 | 13 | 3 | 3 | 3 | 1 | 2 | 0 | 0 |
| ## 2.7 | 12 | 10 | 8 | 11 | 8 | 4 | 1 | 1 | 2 | 0 |
| ## 5.3 | 10 | 11 | 8 | 6 | 6 | 5 | 4 | 2 | 1 | 1 |

¹ Most functions for multivariate techniques need the data in this format - rows = samples, columns = species.

For our analyses we will use two packages:

vegan A package for Community Ecology.

BiodiversityR A package for biodiversity, suitability and community ecology analysis²

```
require(vegan)
require(BiodiversityR)
```

² Advisable further reading: Kindt and Coe (2005) freely available at <http://www.worldagroforestry.org/resources/databases/tree-diversity-analysis>

Rarefaction

The total number of individuals per sample can be calculated as the row sums

```
rowSums(abu)
```

```
##      -1 -0.5    0  0.5    1  1.7  2.7  5.3
##      59  62  85  58  55  45  57  54
```

As the total number of individuals varies we will use the rarefaction method to estimate species richness at a sample size of 40 individuals.

```
rarefy(abu, 40, se = TRUE)
```

```
##      -1 -0.5    0    0.5    1    1.7    2.7
## S  8.4757 9.347 4.0496 4.99092 8.3665 7.8767 8.3122
## se 0.6225 0.687 0.7458 0.09492 0.6788 0.3313 0.6962
##      5.3
## S  9.4139
## se 0.6572
## attr(,"Subsample")
## [1] 40
```

As can be seen species richness drops at the outfall, but increases again downstream (Fig. 7.1). Rarefaction curves can be easily created as well as species accumulation curves:

```
rarecurve(abu, sample = 40)
env <- data.frame(dist = rownames(abu))
rankabuncomp(abu, env, 'dist', scale = 'accumfreq')
```

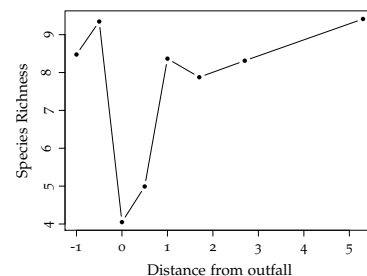


Figure 7.1: Species Richness along outfall

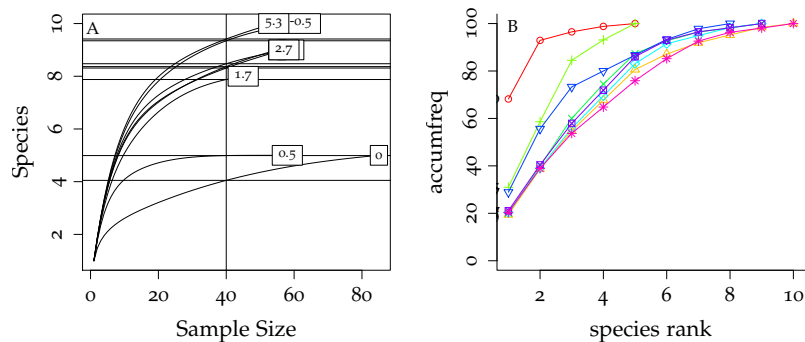


Figure 7.2: A) Rarefaction Curve. B) Rank abundance curve

7.2 Species Diversity

Function `diversity()` from the `vegan` package can calculate the Shannon and Simpson diversity indices ³:

```
diversity(abu, base = 2)

##      -1   -0.5      0    0.5      1    1.7    2.7    5.3
## 2.867 2.986 1.248 2.112 2.789 2.572 2.810 3.016

diversity(abu, index = "simpson")

##      -1   -0.5      0    0.5      1    1.7    2.7
## 0.8486 0.8569 0.4714 0.7485 0.8377 0.7980 0.8415
##      5.3
## 0.8615
```

Brillouin's index is not implemented so have to calculate it by hand:

```
N <- rowSums(abu)
log(factorial(N)/apply(factorial(abu), 1, prod), base = 2)/N

##      -1   -0.5      0    0.5      1    1.7    2.7    5.3
## 2.557 2.658 1.145 1.928 2.471 2.241 2.499 2.653
```

Or use the function `brillouin` from the `qetx` package, which runs the two above lines of code for us:

```
brillouin(abu, 2)
```

Species Evenness

Pielou's J can be easily calculated from the shannon index by dividing it with the number of species in each sample:

```
diversity(abu)/log(rowSums(abu > 0))

##      -1   -0.5      0    0.5      1    1.7    2.7
## 0.9045 0.8989 0.5373 0.9096 0.8799 0.8574 0.8865
##      5.3
## 0.9079
```

7.3 Dissimilarity indices

7.4 Ordination techniques - PCA, RDA, NMDS, MDS, dbRDA

³ The logarithm with base 2 is used for the Shannon index

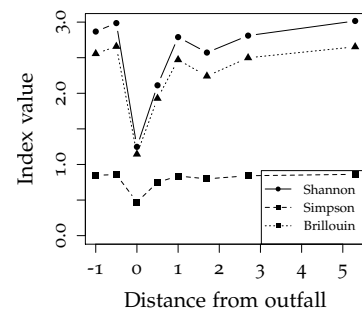


Figure 7.3: Diversity indices along outfall

7.5 Testing hypotheses - PERMANOVA

7.6 Analyzing mesocosm data

Example data

Here we will analyze the pyrifos data set from [Van den Brink and Ter Braak \(1999\)](#) which is shipped with the vegan package.

Describe experiment

```
require(vegan)
data(pyrifos)
head(pyrifos[, c(1:8)])
```

| ## | | Simve | Daplo | Cerpu | Alogu | Aloco | Alore | Aloaf | Copsp |
|----|--------|-------|-------|-------|-------|-------|-------|-------|-------|
| ## | w.4.c1 | 3.951 | 0 | 0 | 0 | 0 | 0 | 0 | 2.773 |
| ## | w.4.c2 | 2.303 | 0 | 0 | 0 | 0 | 0 | 0 | 2.079 |
| ## | w.4.c3 | 4.595 | 0 | 0 | 0 | 0 | 0 | 0 | 3.761 |
| ## | w.4.c4 | 2.398 | 0 | 0 | 0 | 0 | 0 | 0 | 3.296 |
| ## | w.4.c5 | 4.025 | 0 | 0 | 0 | 0 | 0 | 0 | 3.466 |
| ## | w.4.c6 | 2.303 | 0 | 0 | 0 | 0 | 0 | 0 | 2.197 |

So rows correspond to samples and columns are the species (with abbreviated names), a usual species x sites matrix. The column names code treatment and time, but we will create a separate data.frame with information about experimental ditch, sampling time and treatment:

```
ditch <- gl(12, 1, length = 132)
week <- gl(11, 12, labels = c(-4, -1, 0.1, 1, 2, 4, 8, 12,
  15, 19, 24))
dose <- factor(rep(c(0.1, 0, 0, 0.9, 0, 44, 6, 0.1, 44,
  0.9, 0, 6), 11))
pyrifos_env <- data.frame(ditch, week, dose)
```

Introduction

Principle Response Curves (PRC)⁵ are commonly used for analyzing ecotoxicological mesocosm experiments. PRC analyses the change of a community due to a treatment over time and is a special form of Redundancy Analysis (RDA)⁶.

⁵ Van den Brink and Ter Braak, 1999

⁶ Legendre and Legendre, 2013

Overall pattern

With this a hand we can easily calculate and plot (Figure 7.4)⁷ the PRC using the prc() function:

⁷ Note that only species with scores greater or smaller than 1 are displayed to avoid cluttering of the plot

```
pyrifos_prc <- prc(response = pyrifos, treatment = dose,
  time = week)
pyrifos_prc_sum <- summary(pyrifos_prc, scaling = 1)
```

```
plot(pyrifos_prc, select = abs(pyrifos_prc_sum$sp) > 1,
  scaling = 1)
```

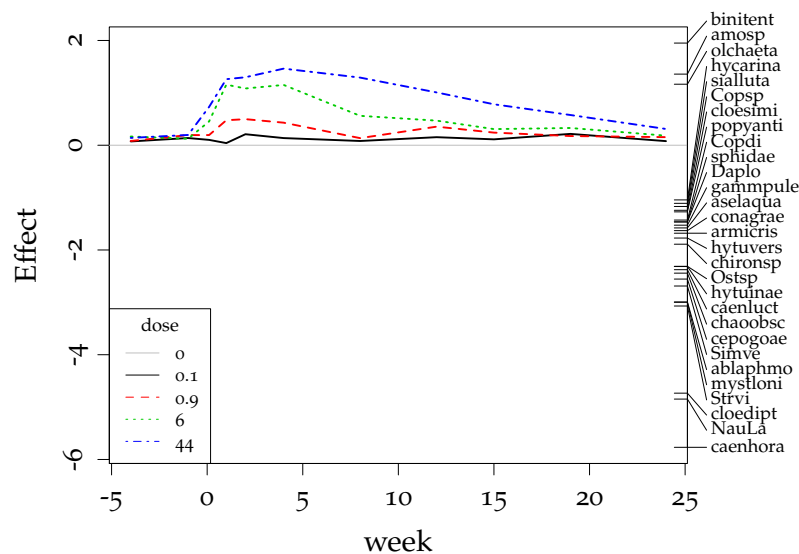


Figure 7.4: Principal response curves (PRC) with species weights for the pyrifos data set indicating effects of the insecticide on the invertebrate community.

The plot shows on the x axis the time and on the y-axis the difference from the control treatments. The farther apart from the x-axis the more different are the communities compared to the control (you can say the x axis represents the control).

We see a clearly treatment-related effect: After application at week 0 the treated communities rapidly change treatment dependent. However to the end of the experiment the treated and the control get similar again, which indicates a 'recovery'.

On the right-hand side we see the species names and their scores. The more extreme the scores the more this species contributed to the plotted pattern. However, you cannot directly infer from these species scores which species are more susceptible. For example *Gammarus pulex* (gammupule) has a relatively low scores, although it's response pattern (Figure 7.5) shows a strong response, but with no recovery. PRC displays global pattern in the community, but the pattern of *G. pulex* is different from most other species, therefore it gets a

lower species score.

We can also look at the numerical output⁸ for this plot using the summary method:

```
pyrifos_prc_sum
```

```
##
## Call:
## prc(response = pyrifos, treatment = dose, time = week)
## Species scores:
##      Simve      Ostsp      NauLa      Strvi binitent caenhora
##      -2.688    -2.312    -4.847    -3.070     1.951    -5.768
## caenluct cloedipt hytuinae ablapmo cepogoe chaoobsc
##      -2.376    -4.734    -2.316    -2.993    -2.555    -2.442
## mystloni
##      -2.998
##
## Coefficients for dose + week:dose interaction
## which are contrasts to dose 0
## rows are dose, columns are week
##           -4      -1      0.1      1      2      4
## 0.1 0.07218 0.1375 0.1020 0.04068 0.2101 0.1364
## 0.9 0.08106 0.1935 0.1936 0.47699 0.4977 0.4306
## 6   0.16616 0.1232 0.4539 1.15638 1.0835 1.1511
## 44  0.13979 0.1958 0.7308 1.26088 1.2978 1.4627
```

The output of prc() gives us more detailed information about the RDA model:

```
pyrifos_prc
```

```
## Call: prc(response = pyrifos, treatment = dose,
## time = week)
##
##              Inertia Proportion Rank
## Total          288.992      1.000
## Conditional     63.349      0.219   10
## Constrained     96.684      0.335   44
## Unconstrained  128.959      0.446   77
## Inertia is variance
##
## Eigenvalues for constrained axes:
##  RDA1  RDA2  RDA3  RDA4  RDA5  RDA6  RDA7  RDA8
```

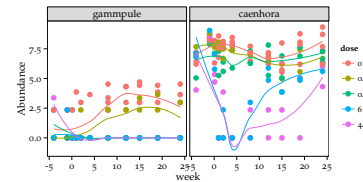


Figure 7.5: Responses of *G. pulex* and *C. horaria* to chlorpyrifos.

⁸ Only a shortened output is given here.

```
## 25.28  8.30  6.04  4.77  4.15  3.86  3.59  3.33
##
## Eigenvalues for unconstrained axes:
##   PC1   PC2   PC3   PC4   PC5   PC6   PC7   PC8
## 17.16  9.19  7.58  6.06  5.73  4.84  4.52  4.10
## (Showed only 8 of all 77 unconstrained eigenvalues)
```

We see that 21.9 % of the variance can be attributed to time (Conditional), 33.5 % can be explained by the treatment regime (Constrained) and 44.6 % of residual variance (Unconstrained), which cannot be explained by time and treatment.

The first RDA axis has an eigenvalue of 25.3. If we divide this eigenvalue by the sum of all eigenvalues, we get the proportion of explained variance which is displayed on the first axis⁹:

```
pyrifos_prc$CCA$eig[1]/sum(pyrifos_prc$CCA$eig) * 100
## RDA1
## 26.15
```

The significance of the PRC diagram can be tested via permutations. However observations from a experimental ditch are not independent, since the same ditch was measured repeatedly during the experiment. We have to take this into account: each ditch represents a time-series. We will permute the whole series of one ditch, keeping the temporal order (see Tab. 7.1).

To setup such a permutation scheme we use the permute package, which is automatically loaded with vegan:

```
control = how(plots = Plots(strata = ditch, type = "free"),
              within = Within(type = "none"), nperm = 99)
```

With this setup we can create a permutation matrix. Each row therein is one permutation, the values are the row numbers of the original data set.

```
set.seed(1234)
permutations <- shuffleSet(nrow(pyrifos), control = control)
```

This can be passed to `permutest`, testing the first eigenvalue of our model.¹⁰

```
mod_perm <- permutest(pyrifos_prc,
                     permutations = permutations,
                     first = TRUE)
mod_perm
```

⁹ `rda()` (and therefore also `prc()`) returns a huge object with all kind of information stored in it. See `?cca.object` for the internal structure. Here I directly access the eigenvalues from this object

Table 7.1: 3 ditches observed for 4 weeks and a possible permutation.

| Week | Ditch | Perm |
|------|-------|------|
| 1 | 1 | 3 |
| 2 | 1 | 3 |
| 3 | 1 | 3 |
| 4 | 1 | 3 |
| 1 | 2 | 1 |
| 2 | 2 | 1 |
| 3 | 2 | 1 |
| 4 | 2 | 1 |
| 1 | 3 | 2 |
| 2 | 3 | 2 |
| 3 | 3 | 2 |
| 4 | 3 | 2 |

This sets up our permutation scheme:

plots We will permute ditches, without any restrictions.

within But within one ditch there will be no permutations.

nperm We want 99 permutations.

¹⁰ `vegan` is in active development and at the moment the `permute`-package isn't fully hooked up. Therefore we have to create a permutation matrix beforehand. In the future we will be able to pass the permutation scheme directly into `vegan` functions.

```
##
## Permutation test for rda
##
## Call: prc(response = pyrifos, treatment = dose,
## time = week)
## Permutation test for first constrained eigenvalue
## Pseudo-F: 15.1 (with 1, 77 Degrees of Freedom)
## Significance: 0.01
## Based on 99 permutations under reduced model.
```

We see that our first axis explains a statistically significant proportion of variation (Fig. 7.6). The minimum p-value that we could get is 0.01 (=1/no. of permutations).

Effects per week

After looking at the overall treatment effect, we may want to look at effects at individual time-points. We follow here ¹¹ and use the ln-transformed nominal dose as continuous explanatory variable ¹².

```
dose_c <- log(20 * as.numeric(levels(dose))[dose] + 1)
```

No we can write a for-loop and compute for every week a RDA and a permutation test ¹³.

```
rdas <- NULL
for (i in levels(week)) {
  rdas[[i]]$rda <- rda(pyrifos[week == i, ] ~
    dose_c[week == i])
  rdas[[i]]$anova <- anova(rdas[[i]]$rda, by = 'terms',
    step = 199)
}
```

However there is also convenience function in the qetx package:

```
rdas <- rda_per_time(pyrifos, dose_c, week)
```

This returns a very big list: one list entry per week and each entry itself contains two lists: rda(RDA-Model) and anova (permutation test)).

From this we have to extract the information we need. We can use `sapply()` to apply a function to every list entry and return results in a vector.

For example to extract the p-values for each week we can use:

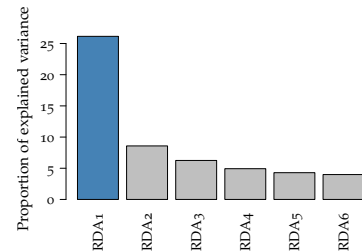


Figure 7.6: Proportion of explained variance of the first 6 RDA-axes. Note that treatment and time were factors and (internally) dummy-coded, therefore we have a total of 44 axes.

¹¹ Van den Brink and Ter Braak, 1999

¹² But before we have to convert dose from a factor to a numeric vector via `as.numeric(levels(x))[x]`

¹³ First we create an empty object (rdas) that will hold our results. Next we run on a subset of data (based on week) a RDA and permutation test. The results of both are stored as a list entry.

```
sapply(rdas, function(x) x$anova[1, 5])
```

| | | | | | | |
|----|----------|----------|----------|----------|----------|----------|
| ## | -4 | -1 | 0.1 | 1 | 2 | 4 |
| ## | 0.437186 | 0.894472 | 0.005025 | 0.000000 | 0.000000 | 0.000000 |
| ## | 8 | 12 | 15 | 19 | 24 | |
| ## | 0.000000 | 0.000000 | 0.030151 | 0.010050 | 0.185930 | |

Have a look at the object structure to write a custom function to extract the information you need:

```
str(rdas[[1]]$anova)
```

Other methods

Other methods to analyse mesocosm experiments include:

multivariate GLMs ¹⁴ In R: mvabund-package.

¹⁴ Warton et al., 2011; and Wang et al., 2012

trait-based indicators ¹⁵ Currently no package, but look at rspear-package.

¹⁵ Liess and Beketov, 2011

community endpoints ¹⁶ Use vegan for computations.

¹⁶ Sanchez-Bayo and Goka, 2012

7.7 Species Sensitivity Distributions

```
require(fitdistrplus)
# or
require(drc)
```


R Session Info

```
sessionInfo()

## R version 3.0.2 (2013-09-25)
## Platform: x86_64-pc-linux-gnu (64-bit)
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8
##  [2] LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8
##  [4] LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8
##  [6] LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8
##  [8] LC_NAME=C
##  [9] LC_ADDRESS=C
## [10] LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8
## [12] LC_IDENTIFICATION=C
##
## attached base packages:
##  [1] stats4      splines      grid          stats          graphics
##  [6] grDevices  utils        datasets      methods        base
##
## other attached packages:
##  [1] drc_2.3-7          plotrix_3.5-1
##  [3] nlme_3.1-111       magic_1.5-4
##  [5] abind_1.4-0        gtools_3.1.0
##  [7] alr3_2.0.5         car_2.0-19
##  [9] nnet_7.3-7         MASS_7.3-29
## [11] fitdistrplus_1.0-1 survival_2.37-4
## [13] ggplot2_0.9.3.1    reshape2_1.2.2
## [15] tikzDevice_0.6.3   filehash_2.2-1
## [17] qetx_0.0.1         vegan_2.1-35
## [19] lattice_0.20-24    permute_0.7-4
```

```
## [21] knitr_1.5
##
## loaded via a namespace (and not attached):
## [1] codetools_0.2-8    colorspace_1.2-4
## [3] dichromat_2.0-0    digest_0.6.3
## [5] evaluate_0.5.1     formatR_0.9
## [7] gtable_0.1.2       highr_0.2.1
## [9] labeling_0.2        munsell_0.4.2
## [11] parallel_3.0.2     plyr_1.8
## [13] proto_0.3-10       RColorBrewer_1.0-5
## [15] scales_0.2.3       stringr_0.6.2
## [17] tools_3.0.2
```

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