Correlated Data and Mixed models

FW8051 Statistics for Ecologists

Department of Fisheries, Wildlife and Conservation Biology

Learning objectives: Correlated Data / Mixed models Overview

- Understand some relatively simple ways to deal with correlated data (bootstrap, Generalized Estimating Equations [later])
- Be able to identify when to use a mixed model
- Learn how to implement mixed models in R/JAGs
  - When the response is Normally distributed (linear mixed effect models, lme)
  - For count, presence absence data (generalized linear mixed effect models, glmms)
  - Understand why generalized linear mixed effects can be difficult to fit
- Be able to describe models and their assumptions using equations and text and match parameters in these equations to estimates in computer output.

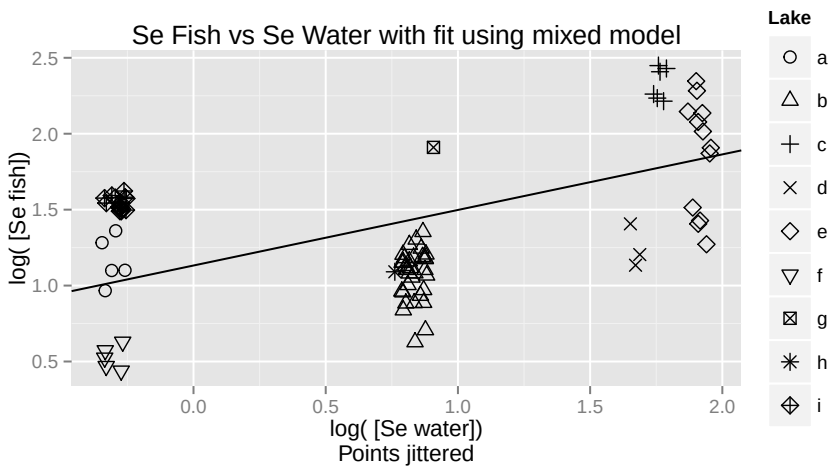
Selenium and Fish

[1]

Selenium, Se, a bi-product of burning coal is measured in...

- A set of 9 lakes
- 1 to 34 fish in each lake (total of 83 observations)

Goal: determine the relationship between mean (log) Se in lake and mean (log) Se in fish.



[1] http://appvoices.org/tag/appalachian-voices/page/7/

Selenium Example

What are the consequences of ignoring the fact that we have multiple observations from each lake?

- If we use linear regression (assuming independence), our SE's will be too small (observations from the same lake are not independent)

What strategies might we use to analyze these data?

Note: our main question involves a predictor-response relationship in which the predictor is constant within each cluster or sample unit

Correlated Data: Folklore Theorem: regression parameter estimators will be unbiased, but standard errors will be too small (if we assume data are independent)

- Can use cluster-level bootstrap for inference
- Generalized estimating equations (that treat clusters as independent sample units. . . more later)

These above methods are most appropriate when:

- The degree of missingness (or amount of data for each cluster) is 'completely random' (we don't have more data from lakes where Se seems to be having a bigger impact on fish)
- We are interested in the effects of covariates to do not vary within a cluster (rather than relationships within particular lakes)
- Correlation is just a nuisance (we don't care about within-lake variability in Se measurements)

Selenium Example

Strategies:

- Fit a linear regression model, but use a cluster-level bootstrap for inference
- Calculate averages of Y for each cluster, then fit linear regression models to these averages (will have 1 observation per cluster)
- Use a mixed model with a random intercept for each cluster (i.e., lake)

Lets do this! See `Se-lake.R`

When to use a mixed model

When you have more than one measurement on the same observational unit

- Multiple observations per lake, animal, study site, etc.

Experiments or surveys with multiple sizes of sample units

- Split-plot designs (treatments applied to whole plots and subplots)
- Cluster samples (samples of households, individuals within households)

When you want to generalize to a larger population of sample units

- Fixed effects: allow inference to only the sample units in the data set
- Random effects: allow us to generalize to a population of sample units by assuming regression parameters have a distribution

Multi-level, Mixed Effects, or Hierarchical models

Key features:

- Regression parameters vary by cluster (e.g., population, individual animal, etc.)
- Regression parameters are assumed to follow a probability distribution
- We estimate the variance of these parameters across clusters

Why are they so popular:

- Provide a framework for modeling correlated and nested data
- Allow estimation of variance components (e.g., variance among individuals, within-individuals)
- Many ecological data sets are hierarchically structured data (e.g., wolves in packs, populations)

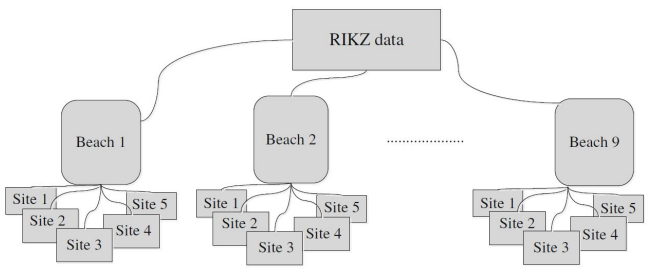


Fig. 5.1 Set up of the RIKZ data. Measurements were taken on 9 beaches, and on each beach 5 sites were sampled. Richness values at sites on the same beach are likely to be more similar to each other than to values from different beaches

Linear regression assumes that observations are independent. Is that reasonable in this case?

- 2 observations from the same beach may be more alike than 2 observations taken from 2 different beaches.
- $\Rightarrow$ observations from the same beach are likely correlated

RIKZ data

Sampling Effort:

- 9 beaches (high, medium, low exposure)
- 5 stations at each beach.

Interest lies in modeling:

- Richness = species richness (number of species counted).

Using macro-fauna and abiotic variables:

- Exposure = low or high exposure to waves, length of surf zone, slope, grain size, and depth of the anaerobic layer
- NAP = height of the sampling station compared to mean tidal level

Multi-level model

Think of models at 2 levels:

- Level 1: model the how individual observations vary within a cluster
- Level 2: model how (cluster-specific) parameters, in the level-1 model, vary (across clusters)

2-stage multi-level modeling approach

Stage 1 (level 1 model):

- Build a separate model for each cluster (beach)
- Only consider variables that are NOT constant within a cluster

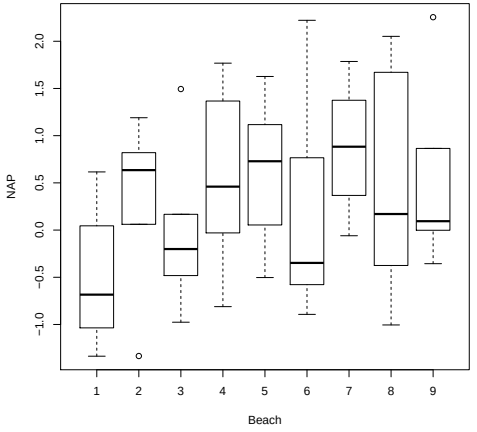
Stage 2 (level 2 model):

- Treat the coefficients from stage 1 as 'data'
- Model the coefficients as a function of variables that are constant within a cluster

Can be useful exploratory approach when you have lots of data for each cluster, but few clusters (e.g., animal telemetry studies)

RIKZ data

`NAP` is a "level-1" covariate (it varies within each cluster)



RIKZ data

`exposure` is a "level-2" covariate (it is constant within a cluster)

```
xtabs(~ exposure + Beach, data=RIKZ)
```

```
        Beach
exposure 1 2 3 4 5 6 7 8 9
       8  0 5 0 0 0 0 0 0 0
       10 5 0 0 0 5 0 0 5 5
       11 0 0 5 5 0 5 5 0 0
```

```
# Only 1 beach with lowest exposure level: modify to have 2 categories
RIKZ$exposure.c<-"High"
RIKZ$exposure.c[RIKZ$exposure%in%c(8,10)]<-"Low"
```

2-Stage approach

Let R_{ij} = the species richness for the j^{th} sample on the i^{th} beach (note: we now need two subscripts!)

Level 1 model: model for observations within each cluster (i.e., for each beach)

$$R_{ij} = \beta_{0i} + \beta_{1i} NAP_{ij} + \epsilon_{ij}; \ (j = 1, 2, \ldots, 5 \text{ observations for each Beach})$$

Each beach has its own intercept β_{0i} and slope β_{1i}

Modified R code

```
RIKZ$NAPc = RIKZ$NAP-mean(RIKZ$NAP)
Beta<-matrix(NA, 9,2) # to hold slope and intercepts
Exposure<-matrix(NA,9,1) # to hold exposure level for each beach
for(i in 1:9){
  Mi<-lm(Richness~NAPc, data=subset(RIKZ, Beach==i))
  Beta[i,]<-coef(Mi)
  Exposure[i]<-subset(RIKZ, Beach==i)$exposure.c[1]
}
level1dat<-data.frame(beach=unique(RIKZ$Beach), intercepts=Beta[,1],
                                    slopes.NAP=Beta[,2], exposure=Expos
```

Note: I have centered the NAP variable

- Makes intercept more meaningful = R_{ij} at the mean value of NAP
- Helps avoid numerical problems and identifiability problems due to correlation of $\hat{\beta}_{0i}$ and $\hat{\beta}_{1i}$

This gives us a data frame of coefficients and level-2 predictors for a level-2 model:

```
level1dat
```

```
  beach intercepts slopes.NAP exposure
1     1  10.692614 -0.3718279      Low
2     2  11.893999 -4.1752712      Low
3     3   2.790385 -1.7553529     High
4     4   2.653600 -1.2485766     High
5     5   9.688335 -8.9001779      Low
6     6   3.841864 -1.3885120     High
7     7   2.992969 -1.5176126     High
8     8   4.293257 -1.8930665      Low
9     9   5.263276 -2.9675304      Low
```

tidyverse solution

```
library(tidyverse)
library(broom)
library(tidyr)
```

```
tidy(lm(Richness~NAPc, data=RIKZ))
```

```
# A tibble: 2 x 5
  term        estimate std.error statistic  p.value
  <chr>          <dbl>     <dbl>     <dbl>    <dbl>
1 (Intercept)     5.69     0.620      9.17 1.11e-11
2 NAPc           -2.87     0.631     -4.55 4.42e- 5
```

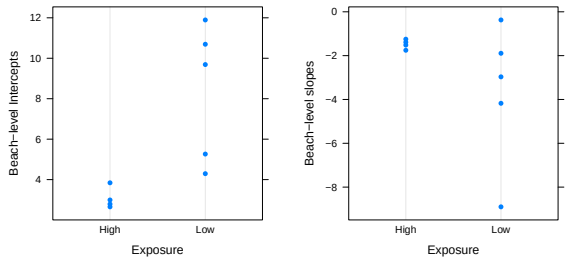
tidyverse solution

```
level1dat.tidy <- RIKZ %>% group_by(Beach) %>%
  do(tidy(lm(Richness~NAPc, data=.))) %>%
  pivot_wider(id_cols=Beach, names_from=term, values_from=estimate)
level1dat.tidy
```

```
# A tibble: 9 x 3
# Groups:   Beach [9]
  Beach `(Intercept)`   NAPc
  <int>         <dbl>  <dbl>
1     1         10.7  -0.372
2     2         11.9  -4.18
3     3          2.79 -1.76
4     4          2.65 -1.25
5     5          9.69 -8.90
6     6          3.84 -1.39
7     7          2.99 -1.52
8     8          4.29 -1.89
9     9          5.26 -2.97
```

Level-2 model

```
library(gridExtra)
library(mosaic)
par(mfrow=c(1,2))
d1<-dotplot(intercepts~Exposure, data=level1dat, xlab="Exposure",
            ylab="Beach-level Intercepts")
d2<-dotplot(slopes.NAP~Exposure, data=level1dat, xlab="Exposure",
            ylab="Beach-level slopes")
grid.arrange(d1, d2, ncol=2)
```



Model for the slope and intercept parameters (analyze the summary statistics, $\hat{\beta}_{0i}, \hat{\beta}_{1i}$) using level-2 predictors (ones that are constant within a cluster)

- $\hat{\beta}_{0i} = \beta_0 + \gamma_0 Exposure_i + b_{0i}$
- $\hat{\beta}_{1i} = \beta_1 + \gamma_1 Exposure_i + b_{1i}$

For now, ignore the fact that the variability of b_{0i}, b_{1i} seems to depend on exposure level ("low", "high").

Level-2 Model: Intercepts

```
summary(lm(intercepts~Exposure, data=level1dat))
```

```
Call:
lm(formula = intercepts ~ Exposure, data = level1dat)

Residuals:
    Min      1Q  Median      3Q     Max
-4.0730 -0.4161 -0.0767  1.3220  3.5277

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)    3.070      1.291   2.378   0.0491 *
ExposureLow    5.297      1.732   3.058   0.0184 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2.582 on 7 degrees of freedom
Multiple R-squared:  0.5719,    Adjusted R-squared:  0.5107
F-statistic: 9.349 on 1 and 7 DF,  p-value: 0.01838
```

Level-2 Model: Slopes

```
summary(lm(slopes.NAP~Exposure, data=level1dat))
```

```
Call:
lm(formula = slopes.NAP ~ Exposure, data = level1dat)

Residuals:
    Min      1Q  Median      3Q     Max
-5.2386 -0.2778  0.0890  0.6940  3.2897

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   -1.478      1.229  -1.202    0.268
ExposureLow   -2.184      1.649  -1.325    0.227

Residual standard error: 2.458 on 7 degrees of freedom
Multiple R-squared:  0.2005,    Adjusted R-squared:  0.08625
F-statistic: 1.755 on 1 and 7 DF,  p-value: 0.2268
```

Putting things together: Composite Equation

Level-1 Model:

- $R_{ij} = \beta_{0i} + \beta_{1i}NAP_{ij} + \epsilon_{ij}$

Level-2 Model:

- $\beta_{0i} = \beta_0 + \gamma_0 Exposure_i + b_{0i}$
- $\beta_{1i} = \beta_1 + b_{1i}$

Substitute into level-1 equation to get the *composite equation*

$$R_{ij} = (\beta_0 + \gamma_0 Exposure_i + b_{0i}) + (\beta_1 + b_{1i})NAP_{ij} + \epsilon_{ij}$$
$$R_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})NAP_{ij} + \gamma_0 Exposure_i + \epsilon_{ij}$$

$\Rightarrow$ *random intercepts and slopes model* (or *random coefficients* model)

Mixed Models

Rather than use a 2-stage approach, we could just posit a model for the data using random and fixed effects.

Random Intercepts Model:

$$R_{ij} = \beta_0 + b_{0i} + \beta_1 NAP_{ij} + \beta_2 Exposure_i + \epsilon_{ij}$$
$$b_{0i} \sim N(0, \tau^2)$$

Random Intercepts and Slopes Model:

$$R_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})NAP_{ij} + \beta_2 Exposure_i + \epsilon_{ij}$$
$$(b_{0i}, b_{1i}) \sim N(0, D)$$

Can think of b_{0i} and b_{1i} as deviations from the average intercept (β_0) and slope (β_1), respectively.

Or, think in terms of beach-level intercepts and slopes: $\beta_{0i} = \beta_0 + b_{0i}$ and $\beta_{1i} = \beta_1 + b_{1i}$, with $(\beta_{0i}, \beta_{1i}) \sim MVN(\beta, D)$

Random intercepts versus random coefficient models

Although random intercepts models are common. . .

Schielzeth and Forstmeier (2009) suggest random slopes are usually appropriate for level-1 predictors (i.e., when x varies within a subject).

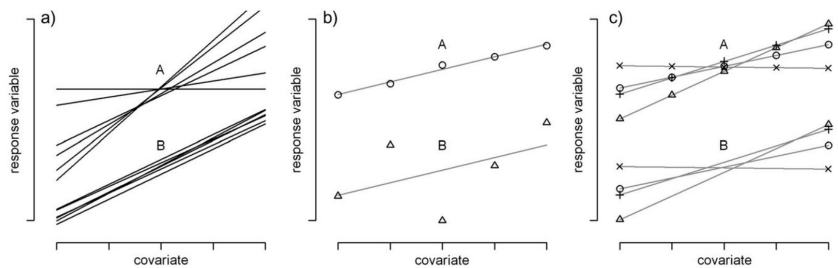


Figure 1
Schematic illustrations of more (A) and less (B) problematic cases for the estimation of fixed-effect covariates in random-intercept models. (a) Regression lines for several individuals with high (A) and low (B) between-individual variation in slopes (σ_s). (b) Two individual regression slopes with low (A) and high (B) scatter around the regression line (σ_r). (c) Regression lines with (A) many and (B) few measurements per individual (independent of the number of levels of the covariate).

See *Readings, Linear Mixed Effects Page* for a copy of Schielzeth and Forstmeier (2009)

Fitting Mixed Effects Models in R

Two popular packages: `nlme` and `lme4`:

`nlme` (older)

- More flexibility for modeling within-cluster correlation and heterogeneity (e.g., time series data, spatial data); see e.g., Ch. 4 in Zuur et al.
- Responses must be Normally distributed

`lme4` (newer)

- Better options for fitting non-normal data: generalized linear mixed effects models [GLMMS] for count or binary data
- Easier to fit non-nested or 'crossed' random effects (e.g., `year` and `Beach` if we had many years of data).
- Cannot handle within-cluster correlation or heterogeneity

Other Packages

Many others too. . . see:
http://glmm.wikidot.com/pkg-comparison

We may also consider:

- glmmADMB
- glmmTMB
- GLMMadaptive

```
summary(lme.fit)
```

```
Linear mixed-effects model fit by REML
  Data: RIKZ
       AIC      BIC    logLik
  240.5538 249.2422 -115.2769

Random effects:
 Formula: ~1 | Beach
        (Intercept) Residual
StdDev:    1.907175 3.059089

Fixed effects:  Richness ~ NAPc + exposure.c
                 Value Std.Error DF   t-value p-value
(Intercept)   3.170680 1.1739988 35  2.700752  0.0106
NAPc         -2.581708 0.4883901 35 -5.286160  0.0000
exposure.cLow  4.532777 1.5755612  7  2.876928  0.0238
 Correlation:
             (Intr) NAPc
NAPc         -0.028
exposure.cLow -0.746  0.037

Standardized Within-Group Residuals:
       Min         Q1        Med         Q3        Max
-1.5163203 -0.4815106 -0.1218701  0.2922855  3.8777562

Number of Observations: 45
Number of Groups: 9
```

```
library(nlme)
lme.fit<-lme(Richness~NAPc+exposure.c, random=~1|Beach, data=RIKZ)
```

fixed effects: `Richness~NAPc+exposure.c`

$$\beta_0 \cdot 1 + \beta_1 NAP_{ij} + \beta_2 exposure.c$$

random effects: `random=~1|Beach`

- 1 is R's notation for the intercept
- |Beach means that the intercepts are random for each Beach.
- Includes a b_{0i} for each Beach (to capture deviations from the fixed, population-level intercept, β_0)

Default assumption: $b_{0i} \sim N(0, \tau^2)$

- We estimate τ^2, not the individual b_{0i}
- Since the b_{0i} are assumed to be "random", we "predict" them (similar to "estimating" errors, ϵ_i using residuals)
- BLUPS = best linear unbiased predictions.

. . . unless you are a Bayesian.

```
summary(lme.fit)$tTable
```

```
                 Value Std.Error DF   t-value      p-value
(Intercept)   3.170680 1.1739988 35  2.700752 1.058924e-02
NAPc         -2.581708 0.4883901 35 -5.286160 6.745464e-06
exposure.cLow  4.532777 1.5755612  7  2.876928 2.375560e-02
```

fixed effects: `Richness~NAPc+exposure.c`

$$\beta_0 + \beta_1 NAP_{ij} + \beta_2 exposure.c$$

Degrees of Freedom (differ for level-1 and level-2 predictors):

- `NAPc` = 35
- `exposure.cLow` = 7

Degrees of Freedom

Level-1: within-subjects degrees of freedom calculated as the number of observations minus the number of groups minus the number of level-1 regressors in the model.

```
nrow(RIKZ) - length(unique(RIKZ$Beach))- 1
```

```
[1] 35
```

Level-2: among-subjects degrees of freedom calculated as the number of groups minus the number of level-2 regressors in the model - 1 for the intercept.

```
length(unique(RIKZ$Beach))- 1 -1
```

```
[1] 7
```

Degrees of Freedom

The formula are not important. . . .what is:

- we have more information about the effect of `NAP` on species richness than `exposure` since `NAP` varies between and within beaches.
- `lme` accounts for the data structure when carrying out statistical tests.

Degrees of Freedom: More accurately

Note: `lme`'s df are essentially correct for balanced data (all clusters have an equal number of observations). For unbalanced data, the tests (and df) are only approximate.

- thus, a decision was made to NOT report p-values for models fit with `lmer` in `lme4`
- there are "better" degrees of freedom approximations for unbalanced data (see, e.g., *lmerTest* package).

Variance Components

```
VarCorr(lme.fit)
```

```
Beach = pdLogChol(1)
            Variance StdDev
(Intercept) 3.637317 1.907175
Residual    9.358027 3.059089
```

$$\epsilon_i \sim N(0, \sigma^2)$$
$$b_{0i} \sim N(0, \tau^2)$$

- $var(\epsilon_{ij}) = \sigma^2 = 9.36$ (variance within a Beach)
- $var(b_{0i}) = \tau^2 = 3.637$ (variance among beaches)

Induced correlation: random intercepts model

$$R_{ij} = \beta_0 + b_{0i} + \beta_1 NAP_{ij} + \beta_2 Exposure_i + \epsilon_{ij}$$

Variance of R_{ij} = $var(b_{0i} + \epsilon_{ij}) = var(b_{0i}) + var(\epsilon_{ij}) = \tau^2 + \sigma^2$

Covariance $(Y_{ij}, Y_{ij'}) = \tau^2$ (2 observations, same cluster [beach] since they share b_{0i})

Covariance $(Y_{ij}, Y_{i'j}) = 0$ (2 observations taken from 2 different clusters [beaches])

Intraclass correlation = $Cor(Y_{ij}, Y_{ij'}) = \frac{\tau^2}{\tau^2+\sigma^2} = 0.28$, correlation among observations taken from the same cluster.

Each beach also has its own intercept. What if we modeled `Beach` using fixed effects?

```
lm.fe <- lm(Richness~factor(Beach)-1+NAPc, data=RIKZ)
summary(lm.fe)
```

```
Call:
lm(formula = Richness ~ factor(Beach) - 1 + NAPc, data = RIKZ)

Residuals:
    Min      1Q  Median      3Q     Max
-4.8518 -1.5188 -0.1376  0.7905 11.8384

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
factor(Beach)1   8.9392     1.4301   6.251 3.61e-07 ***
factor(Beach)2  12.0173     1.3690   8.778 2.29e-10 ***
factor(Beach)3   2.5343     1.3796   1.837 0.074716 .
factor(Beach)4   2.9063     1.3723   2.118 0.041364 *
factor(Beach)5   8.0409     1.3746   5.850 1.22e-06 ***
factor(Beach)6   3.7161     1.3697   2.713 0.010271 *
factor(Beach)7   3.5025     1.3934   2.514 0.016705 *
factor(Beach)8   4.3862     1.3707   3.200 0.002920 **
factor(Beach)9   5.1572     1.3731   3.756 0.000629 ***
NAPc            -2.4928     0.5023  -4.963 1.79e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3.06 on 35 degrees of freedom
Multiple R-squared:  0.8719,    Adjusted R-squared:  0.8353
F-statistic: 23.82 on 10 and 35 DF,  p-value: 9.56e-13
```

Fixed versus random

Fixed effects:

- lm.fe <- lm(Richness~factor(Beach)-1+NAPc, data=RIKZ)
- each beach has its own intercept which we estimate

Random effects:

- lme.fit<-lme(Richness~NAPc+exposure.c, random=~1|Beach, data=RIKZ)
- each beach has its own intercept
- we further assume $\beta_i \sim N(\beta, \sigma^2_{b_{oi}})$ or equivalently $b_{oi} \sim N(0, \sigma^2_{b_{oi}})$
- we estimate the variance of the intercepts and "predict" the beach-level intercepts

Downsides to fixed effects model

- Requires estimation of 8 parameters
- Cannot include `exposure.c` since it is constant for each Beach (and therefore, confounded with the Beach coefficients)

```
lm.fe2 <- lm(Richness~factor(Beach)-1+NAPc+exposure.c, data=RIKZ)
coef(lm.fe2)
```

```
factor(Beach)1 factor(Beach)2 factor(Beach)3 factor(Beach)4 factor(Beach)5 factor(Beach)6
      8.939200      12.017303       2.534266       2.906323       8.040936       3.716094
factor(Beach)7 factor(Beach)8 factor(Beach)9           NAPc  exposure.cLow
      3.502535       4.386168       5.157177      -2.492836             NA
```

- Random coefficients would require interactions between `Beach` and `NAP` (another 8 parameters)

Predicted values

Population Average (averages over beaches):

- $E[R|X] = E(E[R|X, b_{0i}])) = \beta_0 + \beta_1 NAP + \beta_2(\text{exposure="LOW"})$

Subject-Specific (lines for a particular beach):

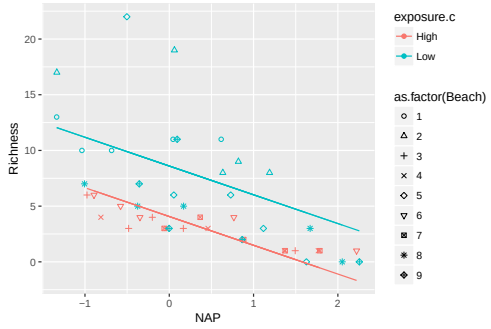
- $E[R|X, b_{0i}] = \beta_0 + b_{0i} + \beta_1 NAP + \beta_2(\text{exposure="LOW"})$

```
# beta0 + beta1*NAP + beta2*Exposure
RIKZ$EY.Pop<-fitted(lme.fit, level=0)

# Subject specific lines
RIKZ$EY.Beach<-fitted(lme.fit, level=1)

head(RIKZ[,13:18],3)
```

```
  Beach Richness exposure.c       NAPc    EY.Pop  EY.Beach
1     1       11        Low -0.3026889  8.484911  9.252313
2     1       10        Low -1.3836889 11.275737 12.043140
3     1       13        Low -1.6836889 12.050250 12.817652
```

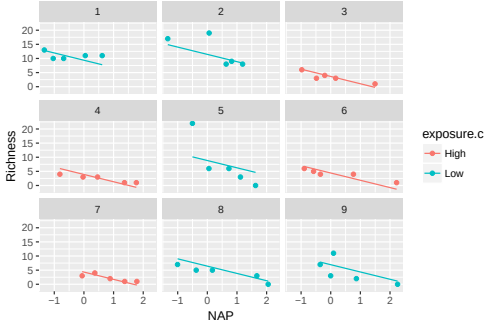
Population Averaged Estimates

```
ggplot(RIKZ, aes(x=NAP, y=Richness, shape=as.factor(Beach),
      colour=exposure.c)) + scale_shape_manual(values=1:9) +
    geom_point() +
    geom_line(aes(y=EY.Pop,x=NAP, color=exposure.c))
```



Subject-specific (Beach-level estimates)

```
ggplot(RIKZ, aes(x=NAP, y=Richness, colour=exposure.c)) +
   facet_wrap(~Beach) + geom_point() +
   geom_line(aes(y=EY.Beach,x=NAP,color=exposure.c))
```



Each beach has its own intercept, but the slopes are the same!

Another alternative: Random intercepts and slopes

$$R_{ij} = \beta_0 + b_{0i} + (\beta_1 + b_{1i})NAP_{ij} + \beta_2 Exposure_i + \epsilon_{ij}$$

$$(b_{0i}, b_{1i}) \sim N(0, D)$$

```
lme.rc<-lme(Richness~NAPc+exposure.c, random=~1+NAPc|Beach, data=RIKZ)
```

`random=~1+NAP|Beach`

- Each beach gets its own intercept, $\beta_0 + b_{0i}$
- Each beach gets its own slope parameter for `NAP`, $\beta_1 + b_{1i}$

```
summary(lme.rc)
```

```
Linear mixed-effects model fit by REML
  Data: RIKZ
       AIC      BIC    logLik
  240.5327 252.6964 -113.2663

Random effects:
 Formula: ~1 + NAPc | Beach
 Structure: General positive-definite, Log-Cholesky parametrization
            StdDev   Corr
(Intercept) 2.179463 (Intr)
NAPc        1.888822 -0.557
Residual    2.549885

Fixed effects:  Richness ~ NAPc + exposure.c
                  Value Std.Error DF   t-value p-value
(Intercept)    3.726341 1.1765068 35  3.167292  0.0032
NAPc          -2.808422 0.7596419 35 -3.697035  0.0007
exposure.cLow  3.704915 1.5176687  7  2.441188  0.0447
 Correlation:
              (Intr) NAPc
NAPc          -0.309
exposure.cLow -0.708  0.024

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-2.02000454 -0.39889890 -0.08147617  0.22318334  2.84753809
```

```
VarCorr(lme.rc)
```

```
Beach = pdLogChol(1 + NAPc)
            Variance StdDev   Corr
(Intercept) 4.750059 2.179463 (Intr)
NAPc        3.567648 1.888822 -0.557
Residual    6.501916 2.549885
```

$$\epsilon_i \sim N(0, \sigma^2)\ (b_{0i}, b_{1,i}) \sim N(0, D);$$
$$D = \begin{bmatrix} var(b_{0i}) & cov(b_{0i}, b_{1i}) \\ cov(b_{0i}, b_{1i}) & var(b_{1,i}) \end{bmatrix}$$

- $var(\epsilon_{ij}) = \sigma^2 = 6.50$ (variance within a Beach)
- $var(b_{0i}) = 4.750$ (variance among beach intercepts)
- $var(b_{1i}) = 3.567$ (variance among beach slopes)
- $\text{Cor}(b_{0i}, b_{1i}) = \frac{Cov(b_{0i}, b_{1i})}{\sqrt{var(b_{0i})var(b_{1i})}} = -0.557$

Predicted Values

Population Average (averages over beaches):

- $E[R|X] = E(E[R|X, b_{0i}])) = \beta_0 + \beta_1 NAP + \beta_2(\text{exposure="LOW"})$

Subject-Specific (lines for a particular beach):

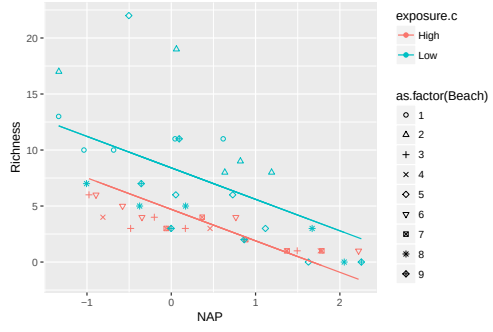
- $E[R|X, b_{0i}] = \beta_0 + b_{0i} + (\beta_1 + b_{1i})NAP + \beta_2(\text{exposure="LOW"})$

```
# beta0 + beta1*NAP + beta2*Exposure
RIKZ$EY.Pop2<-fitted(lme.rc, level=0)

# Subject specific lines
RIKZ$EY.Beach2<-fitted(lme.rc, level=1)

head(RIKZ[,c(13:16, 19:20)],3)
```

```
  Beach Richness exposure.c       NAPc   EY.Pop2 EY.Beach2
1     1       11        Low -0.3026889  8.281334  9.225661
2     1       10        Low -1.3836889 11.317239 11.734620
3     1       13        Low -1.6836889 12.159766 12.430908
```

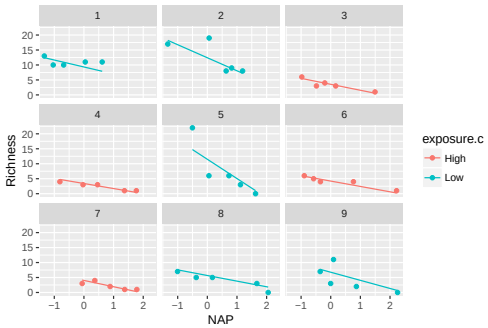
Population Averaged Estimates

```
ggplot(RIKZ, aes(x=NAP, y=Richness, shape=as.factor(Beach), colour=exp
      scale_shape_manual(values=1:9)+geom_point()+
    geom_line(aes(y=EY.Pop2,x=NAP, color=exposure.c))
```



Subject-specific (Beach-level estimates)

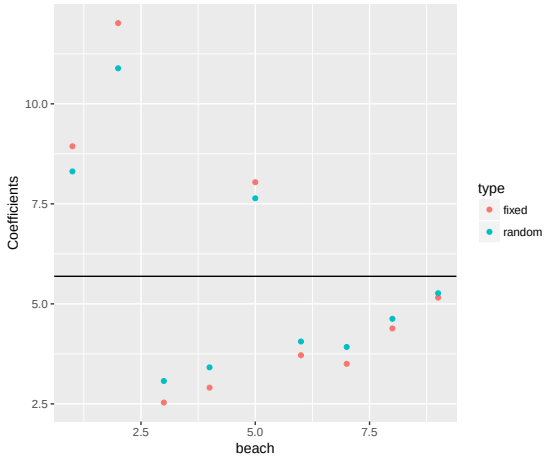
```
ggplot(RIKZ, aes(x=NAP, y=Richness, colour=exposure.c)) +
    facet_wrap(~Beach) + geom_point() +
    geom_line(aes(y=EY.Beach2,x=NAP, color=exposure.c))
```



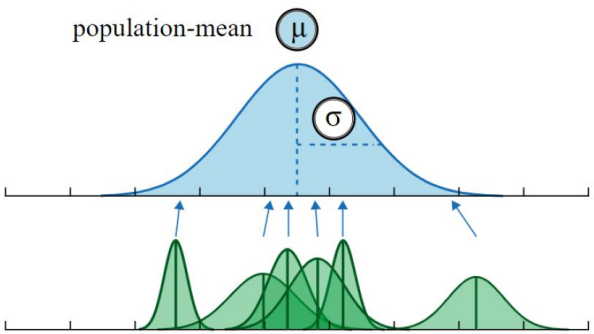
Each beach has its own intercept and slope

Potential Benefit of Mixed Effects Model

Information sharing across beaches: intercepts will be “shrunk” towards the overall population mean:



Shrinkage



https://benediktehinger.de/glm2018/mm_slides.html

Shrinkage depends on:

- how variable the coefficients are across clusters
- the degree of uncertainty associated with individual estimates

```
library(lme4)
lmer.fit<-lmer(Richness~NAPc+exposure.c+(1|Beach), data=RIKZ)
summary(lmer.fit)
```

```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: Richness ~ NAPc + exposure.c + (1 | Beach)
   Data: RIKZ

REML criterion at convergence: 230.6

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.5163 -0.4815 -0.1219  0.2923  3.8778

Random effects:
 Groups   Name        Variance Std.Dev.
 Beach    (Intercept) 3.637    1.907
 Residual             9.358    3.059
Number of obs: 45, groups:  Beach, 9

Fixed effects:
             Estimate Std. Error      df t value Pr(>|t|)
(Intercept)     3.1707     1.1740  6.9478   2.701   0.0308 *
NAPc           -2.5817     0.4884 38.5270  -5.286 5.22e-06 ***
exposure.cLow   4.5328     1.5756  6.9557   2.877   0.0239 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) NAPc
NAPc        -0.028
exposur.cLw -0.746  0.037
```

```
lmer.rc<-lmer(Richness~NAPc+exposure.c+(1+NAPc|Beach), data=RIKZ)
summary(lmer.rc)
```

```
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: Richness ~ NAPc + exposure.c + (1 + NAPc | Beach)
   Data: RIKZ

REML criterion at convergence: 226.5

Scaled residuals:
     Min       1Q   Median       3Q      Max
-2.02001 -0.39890 -0.08148  0.22318  2.84754

Random effects:
 Groups   Name        Variance Std.Dev. Corr
 Beach    (Intercept) 4.750    2.179
          NAPc        3.568    1.889    -0.56
 Residual             6.502    2.550
Number of obs: 45, groups:  Beach, 9

Fixed effects:
             Estimate Std. Error      df t value Pr(>|t|)
(Intercept)     3.7263     1.1765  7.1946   3.167   0.0152 *
NAPc           -2.8084     0.7596  6.4474  -3.697   0.0089 **
exposure.cLow   3.7049     1.5177  5.8357   2.441   0.0515 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) NAPc
NAPc        -0.309
exposur.cLw -0.708  0.024
```

Predicted values lme4

Population level predictions:

```
head(predict(lmer.rc, re.form=~0))
```

```
        1         2         3         4         5         6
8.281335 11.317239 12.159766  6.677726 10.328675  5.065691
```

Beach-level predictions:

```
head(predict(lmer.rc))
```

```
        1         2         3         4         5         6
9.225663 11.734619 12.430907  7.900395 10.917642  7.230622
```

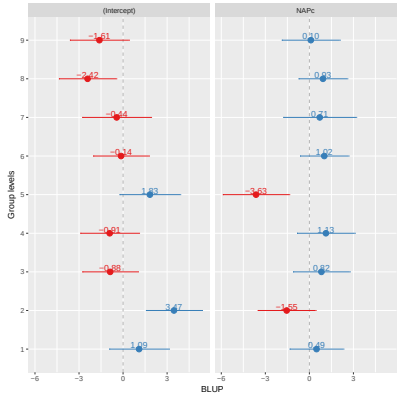
Beach-level predictions (more explicitly:

```
head(predict(lmer.rc, re.form=~(1+NAPc|Beach)))
```

```
        1         2         3         4         5         6
9.225663 11.734619 12.430907  7.900395 10.917642  7.230622
```

Plots of the random effects

```
library(sjPlot)
plot_model(lmer.rc, type="re")
```



Diagnostics

Random Intercepts and Slopes Model:

$$R_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})NAP_{ij} + \beta_2 Exposure_i + \epsilon_{ij}$$
$$(b_{0i}, b_{1i}) \sim N(0, D)$$

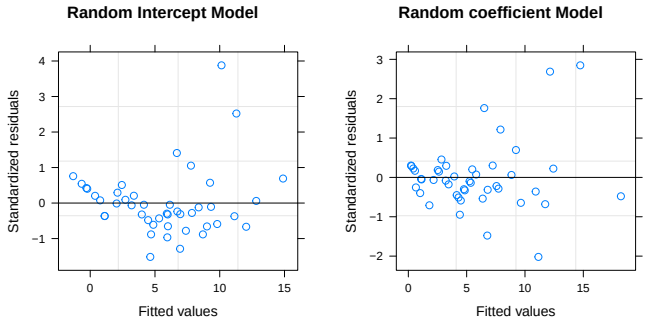
What are our assumptions?

1. Linearity:
   $E[Richness|NAP, Exposure] = \beta_0 + \beta_1 NAP + \beta_p Exposure$
2. Residuals are Normally distributed with constant variance:
   $\epsilon_{ij} \sim N(0, \sigma^2_\epsilon)$
3. Beaches are independent
4. $(b_{0i}, b_{1i}) \sim MVN(0, \Sigma)$, independent of ϵ_{ij}

Residual versus fitted values

Within-beach residuals (ϵ_{ij}) versus fitted values for each beach ($\hat{R}_{ij}$)

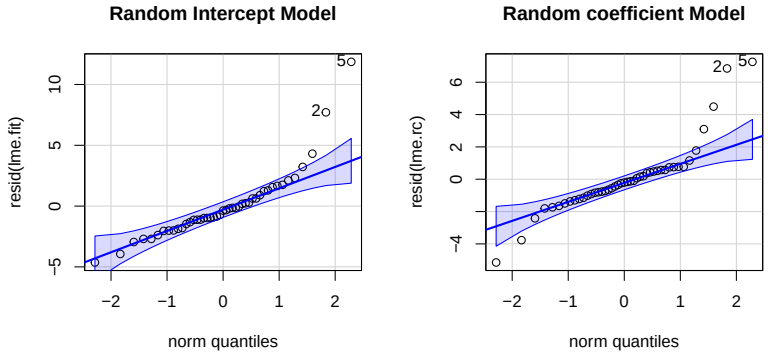
```
ri<-plot(lme.fit, main="Random Intercept Model")
rc<-plot(lme.rc, main="Random coefficient Model")
grid.arrange(ri, rc, ncol=2)
```



Diagnostics

Normality of within-beach errors (ϵ_{ij}):

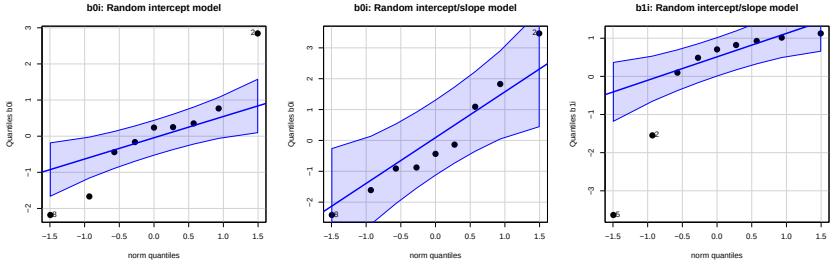
```
library(car)
par(mfrow=c(1,2))
    qqPlot(resid(lme.fit), main="Random Intercept Model")
    qqPlot(resid(lme.rc), main="Random coefficient Model")
```



Diagnostics

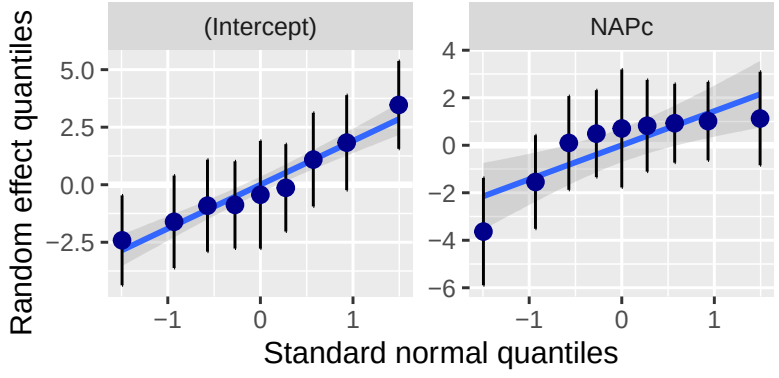
Normality of random effects (b_{oi}, b_{1i})

```
par(mfrow=c(1,3))
    qqPlot(ranef(lme.fit)[,1], cex=1.8,pch=16, ylab="Quantiles b0i",
           main="b0i: Random intercept model") # random intercepts
    qqPlot(ranef(lme.rc)[,1], cex=1.8,pch=16,ylab="Quantiles b0i",
           main="b0i: Random intercept/slope model")  # random interce
    qqPlot(ranef(lme.rc)[,2],cex=1.8,pch=16,ylab="Quantiles b1i",
           main="b1i: Random intercept/slope model")  # random slopes
```



sjPlot library

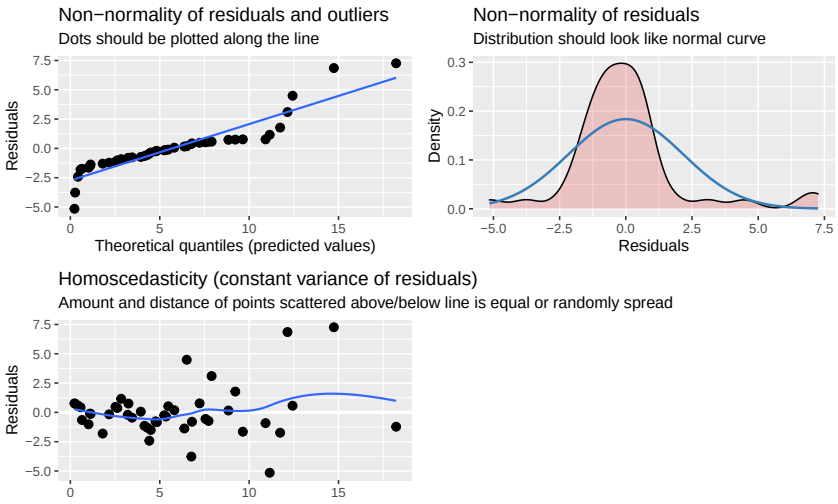
```
p<- plot_model(lmer.rc, type="diag" ) # gives 4 plots
```



sjPlot library

```
p<- plot_model(lmer.rc, type="diag" ) # gives 4 plots
grid.arrange(p[[1]], p[[3]], p[[4]], ncol=2)
```

```
`geom_smooth()` using formula 'y ~ x'
`geom_smooth()` using formula 'y ~ x'
```



Simulation-based testing

See `LectureMixedMods.Rmd` for an option, or have a look at the `RLRsim` or `pbkrtest` packages for simulation-based alternatives.

For nested models, generate a null distribution for likelihood ratio test statistic = - 2(LogL(model1)-LogL(model2)).

- Simulate data from the simpler model
- Fit both models to the simulated data
- Calculate the likelihood ratio statistic
- Repeat many times.

p-value = proportion of simulated observations that are as extreme, or more extreme than the likelihood ratio statistic calculated using the observed data.

Comparing the 2 Models

```
AIC(lme.fit, lme.rc)
```

```
        df      AIC
lme.fit  5 240.5538
lme.rc   7 240.5327
```

This "test" is conservative (tends to overfit) since the variance parameter is "on the boundary" (same goes for Likelihood ratio tests)

See: https://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#testing-significance-of-random-effects

Number of parameters for calculating AIC also depends on focus (on individual subjects or population)

- See: http://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#can-i-use-aic-for-mixed-models-how-do-i-count-the-number-of-degrees-of-freedom-for-a-random-effect

REML versus ML

REML = Restricted Maximum Likelihood (usual default method)

- Variance components estimated using ML are biased high, REML nearly unbiased
- REML maximizes a modified form of the likelihood that depends on the fixed effects components
- Comparisons of models with different fixed effects are not valid when using REML

General Recommendation

- Determine random effects structure by comparing models fit using REML (all w/ the same fixed effects)
- Then, test fixed effects structure using models fit using ML (keeping random effects the same)

For more, see:

- Zuur et al. 5.6

Zuur's Modeling Strategy

Fixed and random effects can "compete" to explain patterns in your response variable...

1. Start with as many covariates in the fixed component as possible
2. Compare models with different random effects structures (via AIC, LR tests). Use method = "REML" and keep fixed component constant.
3. Compare fixed effects models (using AIC, LR tests) using the random structure from step [2]. Use method = "ML"and keep random component constant.
4. Refit the 'best' model from step [4] using method = "REML".
5. Look at diagnostic plots, and modify model as needed

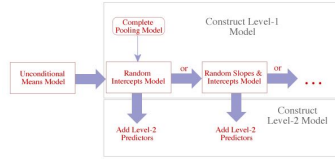


Fig. 1 A general flowchart for fitting multilevel models

Jack Weiss suggests fitting a series of models:

- Pooled model (assuming independence), include level-1 predictors [predictors that vary within clusters] `lm(y~x1)`
- Unconditional means model or variance components model (no predictors, just random intercepts) `lmer(y~1+(1|site))`
- Random intercepts (with level 1 predictors) `lmer(y~x1 + (1|site))`
- Random intercepts and slopes (with level 1 predictors) `lmer(y~x1 + (1+x1|site))`

Pick the best of these, then add level-2 predictors (predictors that are constant within clusters).

Strategy outlined by: Singer, J. D. and Willett, J. B. (2003) Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence. (Oxford University Press, Oxford, UK).

Other

Attempt to make inference from a maximal model:

- Include all random slopes that you can for level 1 predictors
- Simplify as needed when encountering convergence problems.

Lots of debate on how best to approach model building/selection.

Random slopes model will be more conservative

Remember Schielzeth and Forstmeier (2009) suggest random slopes are needed for level-1 predictors (SE increases - see below):

```
# Random intercept
summary(lme.fit)$tTable
```

```
                 Value Std.Error DF   t-value      p-value
(Intercept)   3.170680 1.1739988 35  2.700752 1.058924e-02
NAPc         -2.581708 0.4883901 35 -5.286160 6.745464e-06
exposure.cLow 4.532777 1.5755612  7  2.876928 2.375560e-02
```

```
# Random intercept and slope
summary(lme.rc)$tTable
```

```
                 Value Std.Error DF   t-value      p-value
(Intercept)   3.726341 1.1765068 35  3.167292 0.0031851946
NAPc         -2.808422 0.7596419 35 -3.697035 0.0007425901
exposure.cLow 3.704915 1.5176687  7  2.441188 0.0446809964
```

Marginal Distribution

$$Y_i = X_i\beta + z_i b + \epsilon_i$$
$$\epsilon_i \sim N(0, \Sigma_i)$$
$$b \sim N(0, D)$$

$$Y_i|b \sim N(X_i\beta + Z_i b, \Sigma_i)$$

If we average over (or integrate out) the random effects, we get the marginal Distribution of Y.

$$Y_i \sim N(X_i\beta, V_i), V_i = Z_i D Z_i' + \Sigma_i$$

This is actually what R uses to fit the data.

Marginal model is what R is fitting

For random intercepts model:

$$Y_i \sim N(X_i\beta, V_i)$$

$$V_i = \begin{bmatrix} \sigma^2 & \rho & \cdots & \rho \\ \rho & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & \rho \\ \rho & \cdots & \rho & \sigma^2 \end{bmatrix} \quad \rho = \frac{\tau^2}{\tau^2+\sigma^2}$$

$$\text{Var/Cov matrix for } Y \text{ (all data)} = \begin{bmatrix} V_i & 0 & \cdots & 0 \\ 0 & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & 0 \\ 0 & \cdots & 0 & V_i \end{bmatrix}$$

Fitting the marginal model using `gls`

We might have posited this model directly.

We can fit it using the `gls` function in the `nlme` library

The `gls` function also allows for:

- A variety of assumptions for capturing within-subject correlation
  - ar(1) time series
  - Spatial covariance
- Methods for modeling heterogeneous variance
  - can allow the variance to differ by group (e.g., by exposure level)
  - can allow the variance to depend on a continuous predictor, x (e.g., var = $\sigma^2 x^{2\theta}$)

See Ch 4 Zuur et al. and the section of the course on gls models.

Marginal Model fit using `gls`

```
gls.fit<-gls(Richness~NAPc+exposure.c, method="REML",
             correlation=corCompSymm(form=~1|Beach),
            data=RIKZ)
summary(gls.fit)
```

```
Generalized least squares fit by REML
  Model: Richness ~ NAPc + exposure.c
  Data: RIKZ
       AIC      BIC    logLik
  240.5538 249.2422 -115.2769

Correlation Structure: Compound symmetry
 Formula: ~1 | Beach
 Parameter estimate(s):
      Rho
0.2798938

Coefficients:
                Value Std.Error   t-value p-value
(Intercept)  3.170680 1.1739987  2.700752  0.0099
NAPc        -2.581708 0.4883901 -5.286160  0.0000
exposure.cLow  4.532777 1.5755610  2.876929  0.0063

 Correlation:
              (Intr) NAPc
NAPc          -0.028
exposure.cLow -0.746  0.037

Standardized residuals:
       Min         Q1        Med         Q3        Max
-1.5551728 -0.6415409 -0.1554932  0.4150315  3.3566242

Residual standard error: 3.604905
Degrees of freedom: 45 total; 42 residual
```

```
tab_model(gls.fit, lme.fit, show.r2 = FALSE)
```

	Richness			Richness		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	3.17	0.87 – 5.47	**0.010**	3.17	0.79 – 5.55	**0.011**
NAPc	-2.58	-3.54 – -1.62	**<0.001**	-2.58	-3.57 – -1.59	**<0.001**
exposure.c [Low]	4.53	1.44 – 7.62	**0.006**	4.53	0.81 – 8.26	**0.024**
N				9 Beach		
Observations	45			45		