Correlated Data and Mixed models

FW8051 Statistics for Ecologists

Department of Fisheries, Wildlife and Conservation Biology



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 - 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

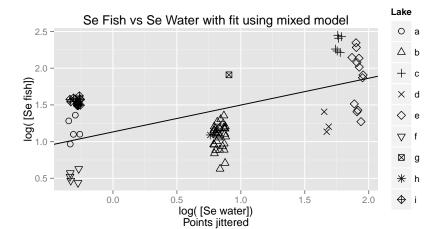


Selenium, Se, a bi-product of burning coal is measured in...

- 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.

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



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

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Lets do this! See Se-lake.R

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- Correlation is just a nuisance (we don't care about within-lake variability in Se measurements)

Mixed models

Models fit using lmer in lme4 package

Model Selection/Model Building Strategies

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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

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Multi-level, Mixed Effects, or Hierarchical models

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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)

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

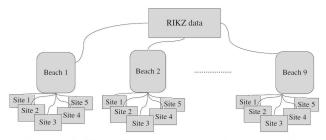


Fig. 5.1 Set up of the RIKZ data. Measurements were taken on 9 beaches, and on each beach 5 sites were sampled. Richness values at sites on the same beach are likely to be more similar to each other than to values from different beaches

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

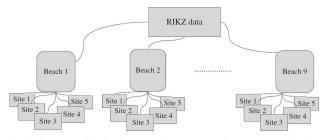


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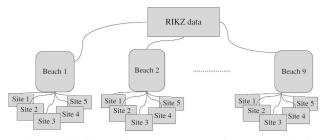


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- 2 observations from the same beach may be more alike than 2 observations taken from 2 different beaches.
- ⇒ observations from the same beach are likely correlated

Multi-level model

Think of models at 2 levels:

• Level 1: model the how individual observations vary within a cluster

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

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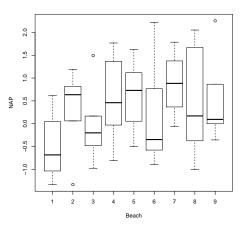
Stage 2 (level 2 model):

- 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)

```
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"</pre>

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!)

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

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

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

Modified R code

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

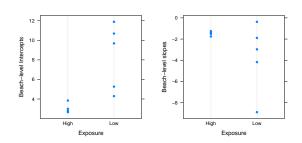
```
library(tidyverse)
library(broom)
library(tidyr)
```

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

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>
           10.7 -0.372
            11.9 -4.18
3
             2.79 -1.76
            2.65 -1.25
5
              9.69 -8.90
6
            3.84 -1.39
            2.99 -1.52
8
              4.29 -1.89
9
              5.26 -2.97
```



Level-2 model

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)

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

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 10 Median 30 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 10 Median 30 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:

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

Level-2 Model:

- $\bullet \ \beta_{0i} = \beta_0 + \gamma_0 Exposure_i + b_{0i}$
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Substitute into level-1 equation to get the *composite equation*

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

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

⇒ random intercepts and slopes model (or *random coefficients* model)

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

Random Intercepts Model:

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

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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).

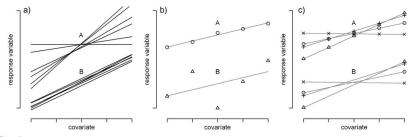


Figure 1 Schematic illustrations of more (A) and less (B) problematic cases for the estimation of fixed-effect covariates in random-intercept models. (a) Regression lines for several individuals with high (A) and low (B) between-individual variation in slopes (σ_b) . (b) Two individual regression slopes with low (A) and high (B) scatter around the regression line (σ_b) . (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)

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- 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

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

fixed effects: Richness~NAPc+exposure.c

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- BLUPS = best linear unbiased predictions.

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- 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)
```

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

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

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

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

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-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
```

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

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 we have more information about the effect of NAP on species richness than exposure since NAP varies between and within beaches.

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.
- 1me accounts for the data structure when carrying out statistical tests.

Degrees of Freedom: More accurately

Note: 1me'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.

Degrees of Freedom: More accurately

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Degrees of Freedom: More accurately

Note: 1me'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)
```

Variance Components

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

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

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

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

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

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

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})

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

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])

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

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

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

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)</pre>
summary(lm.fe)
Call:
lm(formula = Richness ~ factor(Beach) - 1 + NAPc, data = RIKZ)
Residuals:
   Min
         10 Median 30 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

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 $\beta_i \sim N(\beta, \sigma_{b_{oi}}^2)$ or equivalently $b_{oi} \sim N(0, \sigma_{b_{oi}}^2)$
- we estimate the variance of the intercepts and "predict" the beach-level intercepts

• Requires estimation of 8 parameters

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- Cannot include exposure.c since it is constant for each Beach (and therefore, confounded with the Beach coefficients)

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- 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)</pre>
```

```
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
```

coef(lm.fe2)

3.502535

• Requires estimation of 8 parameters

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

4.386168 5.157177

 Cannot include exposure.c since it is constant for each Beach (and therefore, confounded with the Beach coefficients)

```
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
```

-2.492836

NA

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

Predicted values

Population Average (averages over beaches):

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

Predicted values

Population Average (averages over beaches):

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

Subject-Specific (lines for a particular beach):

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

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)</pre>
```

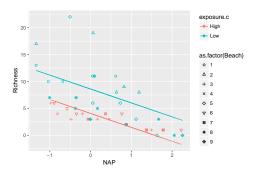
```
    Beach Richness exposure.c
    NAPc
    EY.Pop
    EY.Beach

    1
    1
    1
    Low -0.3026889
    8.484911
    9.252313

    2
    1
    10
    Low -1.3836889
    11.275737
    12.043140

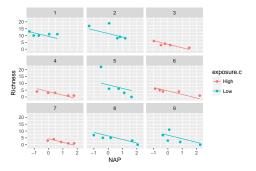
    3
    1
    13
    Low -1.6836889
    12.050250
    12.817652
```

Population Averaged Estimates



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

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

Another alternative: Random intercepts and slopes

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

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

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

 $random = \sim 1 + NAP \mid Beach$

- Each beach gets its own intercept, $\beta_0 + b_{0i}$
- ullet Each beach gets its own slope parameter for NAP, eta_1+b_{1i}

```
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
```

NAPc -0.309 exposure.cLow -0.708 0.024

Residual 2.549885

Standardized Within-Group Residuals:
Min O1 Med

Min Q1 Med Q3 Max -2.02000454 -0.39889890 -0.08147617 0.22318334 2.84753809

VarCorr(lme.rc)

VarCorr(lme.rc)

$$\epsilon_{i} \sim N(0, \sigma^{2}) (b_{0i}, b_{1,i}) \sim N(0, D);$$

$$D = \begin{bmatrix} var(b_{0i}) & cov(b_{0i}, b_{1i}) \\ cov(b_{0i}, b_{1i}) & var(b_{1,i}) \end{bmatrix}$$

VarCorr(lme.rc)

$$\epsilon_i \sim N(0, \sigma^2) \ (b_{0i}, b_{1,i}) \sim N(0, D);$$

$$D = \begin{bmatrix} var(b_{0i}) & cov(b_{0i}, b_{1i}) \\ cov(b_{0i}, b_{1i}) & var(b_{1,i}) \end{bmatrix}$$

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

•
$$\operatorname{Cor}(b_{0i}, b_{1i}) = \frac{\operatorname{Cov}(b_{0i}, b_{1i})}{\sqrt{\operatorname{var}(b_{0i})\operatorname{var}(b_{1i})}} = -0.557$$

Predicted Values

Population Average (averages over beaches):

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

Predicted Values

Population Average (averages over beaches):

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

Subject-Specific (lines for a particular beach):

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

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):

• $E[R|X, b_{0i}] = \beta_0 + b_{0i} + (\beta_1 + b_{1i})NAP + \beta_2$ (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)</pre>
```

```
      Beach Richness exposure.c
      NAPC
      EY.Pop2
      EY.Beach2

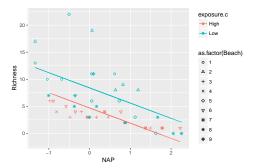
      1
      1
      1
      Low -0.3026889
      8.281334
      9.225661

      2
      1
      10
      Low -1.3836889
      11.317239
      11.734620

      3
      1
      13
      Low -1.6836889
      12.159766
      12.430908
```

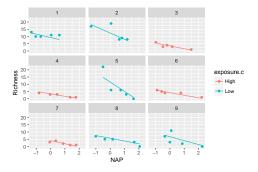
Population Averaged Estimates

```
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)

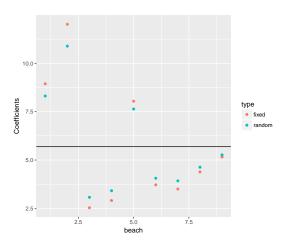
```
ggplot(RIKZ, aes(x=NAP, y=Richness, colour=exposure.c)) +
  facet_wrap(~Beach) + geom_point() +
  geom_line(aes(y=EY.Beach2,x=NAP, color=exposure.c))
```



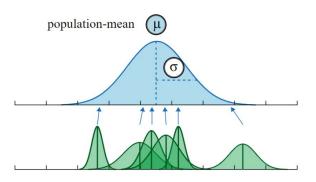
Each beach has its own intercept and slope

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

Mixed models

Models fit using lmer in lme4 package

Model Selection/Model Building Strategies

```
library(lme4)
lmer.fit<-lmer(Richness~NAPc+exposure.c+(1|Beach), data=RIKZ)</pre>
summary(lmer.fit)
Linear mixed model fit by REML ['lmerMod']
Formula: Richness ~ NAPc + exposure.c + (1 | Beach)
  Data: RIKZ
REML criterion at convergence: 230.6
Scaled residuals:
   Min 10 Median 30 Max
-1.5163 -0.4815 -0.1219 0.2923 3.8778
Random effects:
                 Variance Std.Dev.
Groups Name
Beach (Intercept) 3.637 1.907
                  9.358 3.059
Residual
Number of obs: 45, groups: Beach, 9
Fixed effects.
            Estimate Std. Error t value
(Intercept) 3.1707 1.1740 2.701
NAPc -2.5817 0.4884 -5.286
exposure.cLow 4.5328 1.5756 2.877
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 ['lmerMod']
Formula: Richness ~ NAPc + exposure.c + (1 + NAPc | Beach)
    Data: RIKZ</pre>
```

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 t value | (Intercept) | 3.7263 | 1.1765 | 3.167 | NAPC | -2.8084 | 0.7596 | -3.697 | exposure.clow | 3.7049 | 1.5177 | 2.441 |

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))
```

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
```

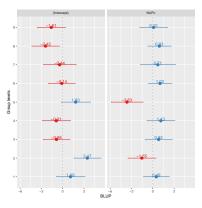
Beach-level predictions (more explicitly:

```
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")
```



Random Intercepts and Slopes Model:

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

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

Random Intercepts and Slopes Model:

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

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

- 1. Linearity: $E[Richness|NAP, Exposure] = \beta_0 + \beta_1 NAP + \beta_p Exposure$
- 2. Residuals are Normally distributed with constant variance: $\epsilon_{ij} \sim N(0, \sigma_{\epsilon}^2)$

Random Intercepts and Slopes Model:

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

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

- 1. Linearity: $E[Richness|NAP, Exposure] = \beta_0 + \beta_1 NAP + \beta_p Exposure$
- 2. Residuals are Normally distributed with constant variance: $\epsilon_{ij} \sim N(0, \sigma_{\epsilon}^2)$
- 3. Beaches are independent

Random Intercepts and Slopes Model:

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

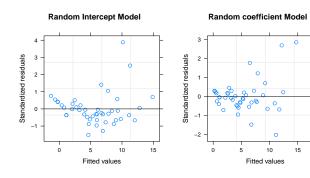
$$(b_{0i}, b_{1i}) \sim N(0, D)$$

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

Residual versus fitted values

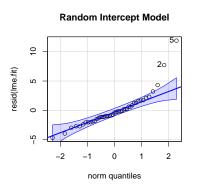
Within-beach residuals (ϵ_{ij}) versus fitted values for each beach (\hat{R}_{ij})

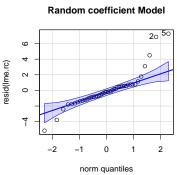
```
ri<-plot(lme.fit, main="Random Intercept Model")
rc<-plot(lme.rc, main="Random coefficient Model")
grid.arrange(ri, rc, ncol=2)</pre>
```



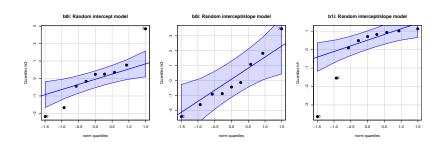
Normality of within-beach errors (ϵ_{ij}):

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



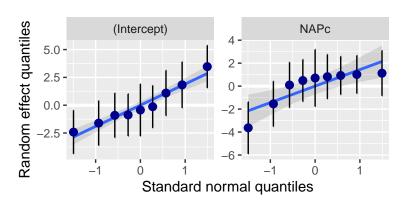


Normality of random effects (b_{oi} , b_{1i})



sjPlot library

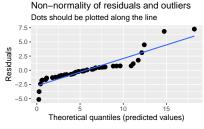
```
p<- plot_model(lmer.rc, type="diag") # gives 4 plots</pre>
```

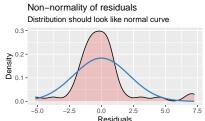


sjPlot library

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

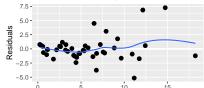
```
'geom_smooth()' using formula 'y \sim x'' 'geom_smooth()' using formula 'y \sim x'
```





Homoscedasticity (constant variance of residuals)

Amount and distance of points scattered above/below line is equal or randomly spread



Mixed models

Models fit using lmer in lme4 package

Model Selection/Model Building Strategies

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/mixed models-misc/glmmFAQ.html # testing-significance-of-random-effects for the property of t

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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

Simulation-based testing

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

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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
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- Repeat many times.

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

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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)

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For more, see:

Zuur et al. 5.6

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

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- 4. Refit the 'best' model from step [4] using method = "REML".
- 5. Look at diagnostic plots, and modify model as needed

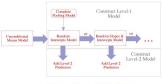


Fig. 1 A general flowchart for fitting multilevel models

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))
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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:

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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

$$Y_i = X_i \beta + z_i b + \epsilon_i$$

$$\epsilon_i \sim N(0, \Sigma_i)$$

$$b \sim N(0, D)$$

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

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

This is actually what R uses to fit the data.

Marginal model is what R is fitting

For random intercepts model:

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

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

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- A variety of assumptions for capturing within-subject correlation
 - ar(1) time series
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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=corCompSvmm (form=~1|Beach),
           data=RIKZ)
summary(gls.fit)
Generalized least squares fit by REML
 Model: Richness ~ NAPc + exposure.c
  Data: RIKZ
      ATC
               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
         -2.581708 0.4883901 -5.286160 0.0000
NAPC
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
                  01
                            Med
                                                 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)

| Predictors | Richness | | | Richness | | |
|------------------|-----------|-------------|--------|-----------|-------------|--------|
| | 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 | -3.541.62 | <0.001 | -2.58 | -3.571.59 | <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 | | |