

Linear Mixed Effects 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])

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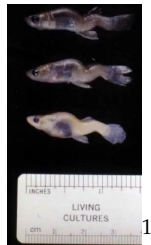
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 - Understand why generalized linear mixed effects can be difficult to fit
- Be able to describe models and their assumptions using equations and text and match parameters in these equations to estimates in computer output.

Selenium and Fish



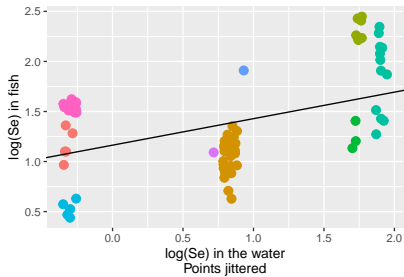
Selenium, Se, a bi-product of burning coal is measured in...

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

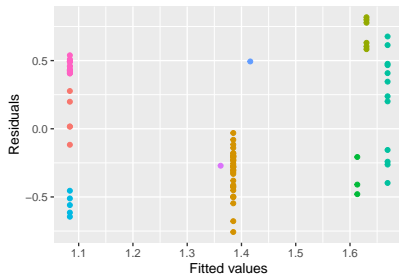
Goal: determine the relationship between mean (log) Se in lake and mean (log) Se in fish.

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

Se in Fish vs Se Water with linear regression line



Residual vs. fitted values



Selenium Example

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

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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

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Strategies:

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Lets do this!

Mixed models

Model Selection/Model Building Strategies

When to use a mixed model

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

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- Cluster samples (samples of households, individuals within households)

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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 cluster-specific regression parameters come from a common distribution

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- Provides a framework for modeling data where the independence assumption is violated

Pines data (book example)

Study objective: investigate tradeoffs between growth rate, size, and lifespan of Mountain pines (*Pinus montana*) in Switzerland (Bigler, 2016).

Is it better to grow quick but die young? Grow more slowly and live longer?

Sample units:

- 160 dead standing trees sampled at 20 sites

Variables:

- dbh = diameter at breast height (size of tree)
- maximum age (i.e., lifespan)
- Aspect of study site

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

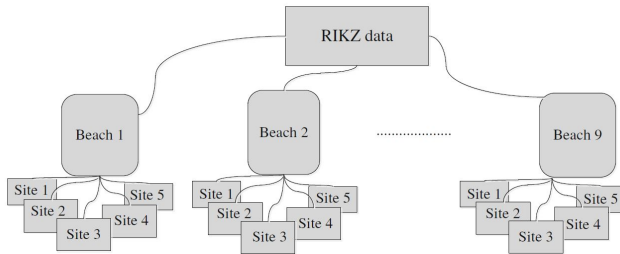


Fig. 5.1 Set up of the RIKZ data. Measurements were taken on 9 beaches, and on each beach 5 sites were sampled. Richness values at sites on the same beach are likely to be more similar to each other than to values from different beaches

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

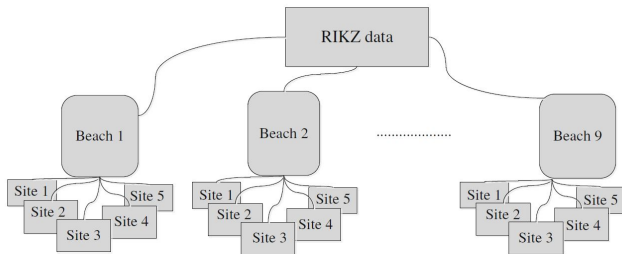


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- 2 observations from the same beach may be more alike than 2 observations taken from 2 different beaches.

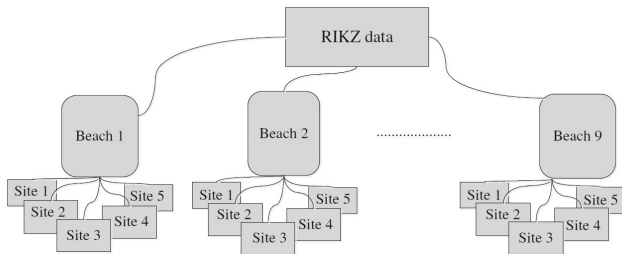


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Linear regression assumes that observations are independent. Is that reasonable in this case?

- 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

Multi-level model

Think of models at 2 levels:

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

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- 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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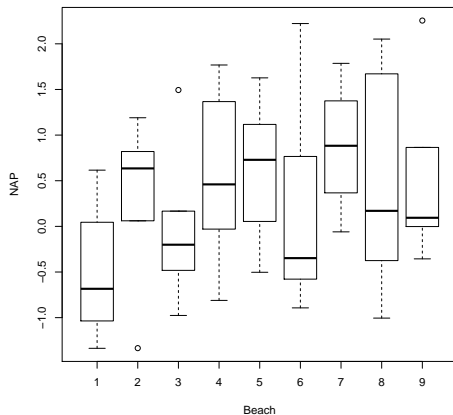
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- Only consider variables that are NOT constant within a cluster

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

NAP is a “level-1” covariate (it varies within each cluster)



RIKZdat

exposure is a “level-2” covariate (it is constant within a cluster)

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

	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  
RIKZdat$exposure.c<-"High"  
RIKZdat$exposure.c[RIKZdat$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!)

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

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

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

Modified R code

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

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 $\hat{\beta}_{1i}$

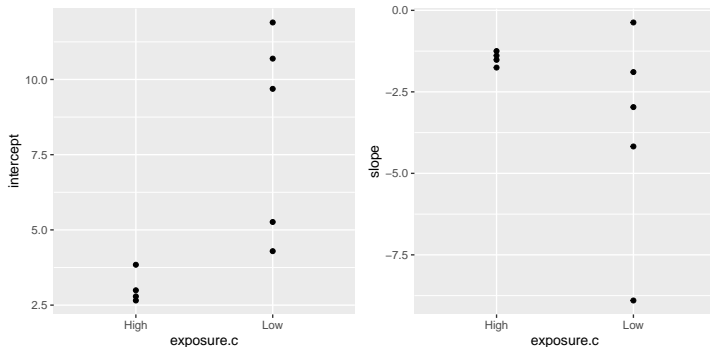
This gives us a data frame of coefficients and level-2 predictors for a level-2 model:

```
betadat
```

	Beach	intercept	slope	exposure.c
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

For a tidyverse solution - see book/R code.

```
library(ggplot2); library(patchwork)
g1 <-ggplot(betadat, aes(exposure.c, intercept)) + geom_point()
g2 <-ggplot(betadat, aes(exposure.c, slope)) + geom_point()
g1+g2
```



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)

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

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(intercept ~ exposure.c, data = betadat))
```

Call:

```
lm(formula = intercept ~ exposure.c, data = betadat)
```

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 *
exposure.cLow	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(slope ~ exposure.c, data = betadat))
```

Call:

```
lm(formula = slope ~ exposure.c, data = betadat)
```

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
exposure.cLow	-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:

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

\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:

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

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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 $(\beta_{0i}, \beta_{1i}) \sim MVN(\beta, D)$

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), but slowly being replaced by other options (e.g., `glmmTMB`, `INLA`);
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`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>

Two others that we will consider:

- glmmTMB
- GLMMadaptive

For now, let's fit the random intercept and random intercept and slope models using the `lmer` function in the `lme4` package!

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

Fit this model in R using `lmer` and identify $\hat{\beta}_0, \hat{\beta}_1, \hat{\sigma}, \hat{\tau}$!

Random Intercepts and Slopes Model:

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

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

Fit this model in R and identify the different parameters:

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Fit this model in R and identify the different parameters:

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

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- BLUPS = best linear unbiased predictions (see Book section 18.8 for details on how they are calculated).

If you are a Bayesian, you can ignore the distinction between “prediction” and “estimation”... ALL parameters are random variables!

Fixed versus Random Comparison

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

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

Call:

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

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=RIKZdat)`
- each beach has its own intercept which we estimate

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Random effects:

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

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

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

Downsides to fixed effects model

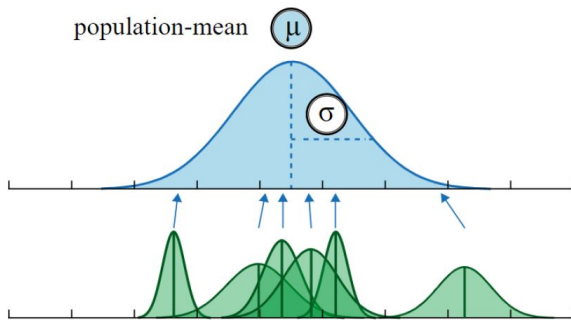
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factor(Beach)8  factor(Beach)9      NAPc  exposure.cLow
      4.386168      5.157177      -2.492836      NA
```

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

Shrinkage (demonstration in R!)



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

Predicted values

Population Average (averages over beaches):

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

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Subject-Specific (lines for a particular beach):

- $E[R|X, b_{0i}] = \beta_0 + b_{0i} + (\beta_1 + b_1)NAP + \beta_2(exposure="LOW")$

R for a demonstration!

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?

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What are our assumptions?

1. Linearity:

$$E[Richness|NAP, Exposure] = \beta_0 + \beta_1 NAP + \beta_p Exposure$$

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3. Beaches are independent

4. $(b_{0i}, b_{1i}) \sim MVN(0, D)$, independent of ϵ_{ij}

Diagnostic plots

- Default plot method: plot of within beach residuals, $\hat{\epsilon}_{ij}$ versus beach-level predictions
$$\hat{R}_{ij} = \hat{\beta}_0 + \hat{b}_{0i} + (\hat{\beta}_1 + \hat{b}_1)NAP + \hat{\beta}_2(exposure = "low")$$
- `check_model` function offers many more checks (see R for a demonstration!)

Degrees of Freedom (lme)

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

Linear mixed-effects model fit by REML

Data: RIKZdat

	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:

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

- $NAP_c = 35$
- `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(RIKZdat) - length(unique(RIKZdat$Beach)) - 1
```

```
[1] 35
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```

```
[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(RIKZdat$Beach)) - 1 - 1
```

```
[1] 7
```

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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.
- `lme` accounts for the data structure when carrying out statistical tests.

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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.

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- 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 and Section 18.12.3 of the book and R code).

Mixed models

Model Selection/Model Building Strategies

Comparing the 2 Models

AIC comparisons and likelihood ratio tests are complicated by the fact that the variance parameter is “on the boundary”

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

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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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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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For more, see:

- Zuur et al. 5.6

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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

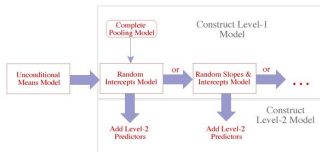


Fig. 1 A general flowchart for fitting multilevel models

Jack Weiss suggests fitting a series of models:

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

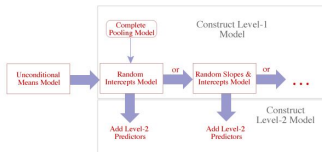


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- Random intercepts and slopes (with level 1 predictors) $\text{lmer}(y \sim x1 + (1 + x1|\text{site}))$

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).

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).

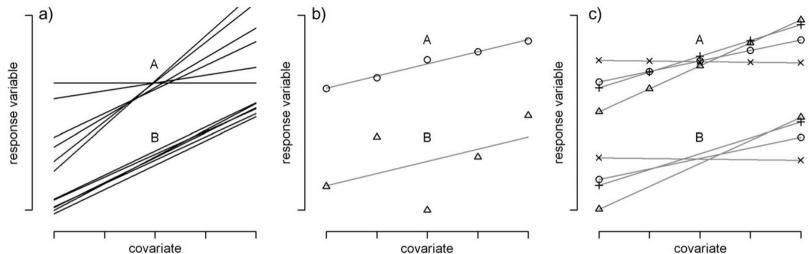


Figure 1

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_β). (b) Two individual regression slopes with low (A) and high (B) scatter around the regression line (σ_ϵ). (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)

Maximal model

Attempt to make inference from a maximal model:

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- Simplify as needed when encountering convergence problems.

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