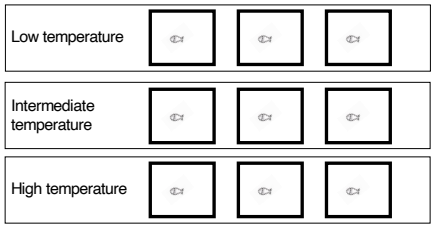
# Understanding mixed models for ANOVAs (mixed model ANOVA or Linear Mixed Effects ANOVA)

1

The advantages of mixed models - increase statistical power and estimation accuracy through dependent replication and design convenience (particularly in observational studies).

Do we need a random effect here?

Effects of temperature on fish growth
(difference in growth begin/end of study)

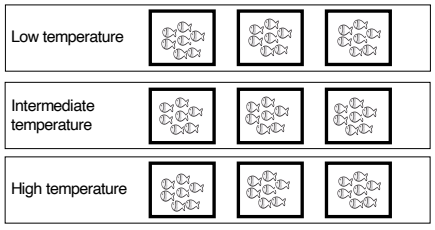



2

The advantages of mixed models - increase statistical power and estimation accuracy through dependent replication and design convenience (particularly in observational studies).

Do we need a random effect here?

Effects of temperature on fish growth
(difference in growth begin/end of study)

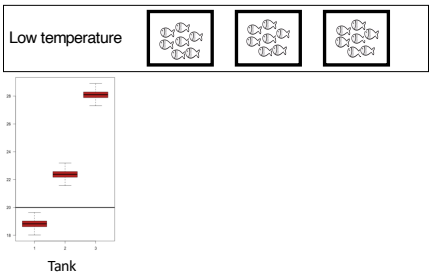



3

The advantages of mixed models - increase statistical power and estimation accuracy through dependent replication and design convenience (particularly in observational studies).

Do we need a random effect here?

Effects of temperature on fish growth
(difference in growth begin/end of study)

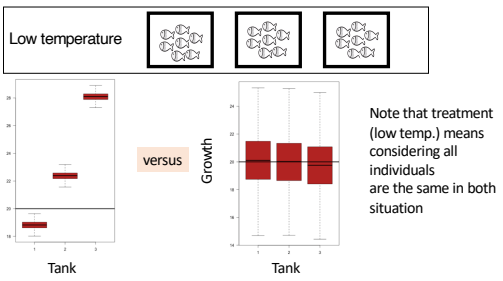



4

The advantages of mixed models - increase statistical power and estimation accuracy through dependent replication and design convenience (particularly in observational studies).

Do we need a random effect here?

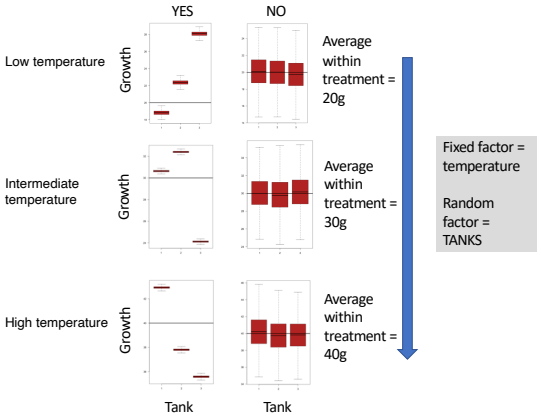
Effects of temperature on fish growth
(difference in growth begin/end of study)




Note that treatment (low temp.) means considering all individuals are the same in both situation

5

Do we need a random effect here? Which experimental results you should trust the most?



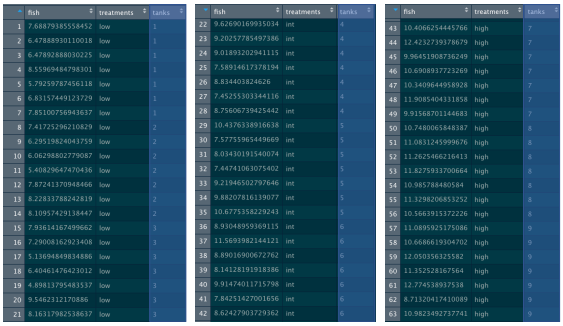

Fixed factor = temperature

Random factor = TANKS

6

Data structure (fixed effect) - in a regular fixed factor ANOVA individual fish would be treated as an individual replicate regardless of tank, i.e., 21 individual fish per temperature treatment (potential reason: put fish in tanks just to reduce logistics). Tank variation is NOT considered.

```
anova(lm(fish ~ treatments))
```

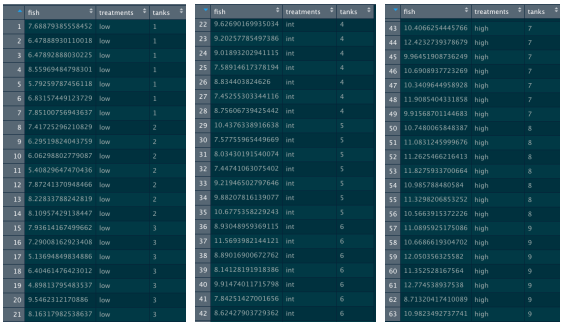


| | fish | treatments | tanks |
|---|---|---|---|
| 1 | 7.68879385558452 | low | 1 |
| 2 | 6.47888930110018 | low | 1 |
| 3 | 6.47892888030225 | low | 1 |
| 4 | 8.55969484798301 | low | 1 |
| 5 | 5.79259787456118 | low | 1 |
| 6 | 6.83157449123729 | low | 1 |
| 7 | 7.85100756943637 | low | 1 |
| 8 | 7.41725296210829 | low | 2 |
| 9 | 6.29519824043759 | low | 2 |
| 10 | 6.06298802779087 | low | 2 |
| 11 | 5.40829647470436 | low | 2 |
| 12 | 7.87241370948466 | low | 2 |
| 13 | 8.22833788242819 | low | 2 |
| 14 | 8.10957429138447 | low | 2 |
| 15 | 7.93614167499662 | low | 3 |
| 16 | 7.29008162923408 | low | 3 |
| 17 | 5.13698489834886 | low | 3 |
| 18 | 6.40461476423012 | low | 3 |
| 19 | 4.89813795483537 | low | 3 |
| 20 | 9.5462312170886 | low | 3 |
| 21 | 8.16317982538637 | low | 3 |
| 22 | 9.62690169935034 | int | 4 |
| 23 | 9.20257785497386 | int | 4 |
| 24 | 9.01893202941115 | int | 4 |
| 25 | 7.58914617378194 | int | 4 |
| 26 | 8.834403824626 | int | 4 |
| 27 | 7.45255303344116 | int | 4 |
| 28 | 8.75606739425442 | int | 4 |
| 29 | 10.4376338916638 | int | 5 |
| 30 | 7.57755965449669 | int | 5 |
| 31 | 8.03430191540074 | int | 5 |
| 32 | 7.44741063075402 | int | 5 |
| 33 | 9.21946502797646 | int | 5 |
| 34 | 9.88207816139077 | int | 5 |
| 35 | 10.6775358229243 | int | 5 |
| 36 | 8.93048959369115 | int | 6 |
| 37 | 11.5693982144121 | int | 6 |
| 38 | 8.89016900672762 | int | 6 |
| 39 | 8.14128191918386 | int | 6 |
| 40 | 9.91474011715798 | int | 6 |
| 41 | 7.84251427001656 | int | 6 |
| 42 | 8.62427903729362 | int | 6 |
| 43 | 10.4066254445766 | high | 7 |
| 44 | 12.4232739178679 | high | 7 |
| 45 | 9.96451908736249 | high | 7 |
| 46 | 10.6908937723269 | high | 7 |
| 47 | 10.3409644958928 | high | 7 |
| 48 | 11.9085404331858 | high | 7 |
| 49 | 9.91568701144683 | high | 7 |
| 50 | 10.7480065848387 | high | 8 |
| 51 | 11.0831245999676 | high | 8 |
| 52 | 11.2625466216413 | high | 8 |
| 53 | 11.8275933700664 | high | 8 |
| 54 | 10.985788480584 | high | 8 |
| 55 | 11.3298206853252 | high | 8 |
| 56 | 10.5663915372226 | high | 8 |
| 57 | 11.0895925175086 | high | 9 |
| 58 | 10.6686619304702 | high | 9 |
| 59 | 12.050356325582 | high | 9 |
| 60 | 11.352528167564 | high | 9 |
| 61 | 12.774538937538 | high | 9 |
| 62 | 8.71320417410089 | high | 9 |
| 63 | 10.9823492737741 | high | 9 |

7

Data structure (mixed effect) – here individual fish are treated as replicates within tanks and tank variation within treatments is also considered; hence we need to use a one-factorial mixed-effects ANOVA:

```
lme(fish ~ treatments, random=~1|tanks)
```



| | fish | treatments | tanks |
|---|---|---|---|
| 1 | 7.68879385558452 | low | 1 |
| 2 | 6.47888930110018 | low | 1 |
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| 4 | 8.55969484798301 | low | 1 |
| 5 | 5.79259787456118 | low | 1 |
| 6 | 6.83157449123729 | low | 1 |
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| 9 | 6.29519824043759 | low | 2 |
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| 16 | 7.29008162923408 | low | 3 |
| 17 | 5.13698489834886 | low | 3 |
| 18 | 6.40461476423012 | low | 3 |
| 19 | 4.89813795483537 | low | 3 |
| 20 | 9.5462312170886 | low | 3 |
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| 34 | 9.88207816139077 | int | 5 |
| 35 | 10.6775358229243 | int | 5 |
| 36 | 8.93048959369115 | int | 6 |
| 37 | 11.5693982144121 | int | 6 |
| 38 | 8.89016900672762 | int | 6 |
| 39 | 8.14128191918386 | int | 6 |
| 40 | 9.91474011715798 | int | 6 |
| 41 | 7.84251427001656 | int | 6 |
| 42 | 8.62427903729362 | int | 6 |
| 43 | 10.4066254445766 | high | 7 |
| 44 | 12.4232739178679 | high | 7 |
| 45 | 9.96451908736249 | high | 7 |
| 46 | 10.6908937723269 | high | 7 |
| 47 | 10.3409644958928 | high | 7 |
| 48 | 11.9085404331858 | high | 7 |
| 49 | 9.91568701144683 | high | 7 |
| 50 | 10.7480065848387 | high | 8 |
| 51 | 11.0831245999676 | high | 8 |
| 52 | 11.2625466216413 | high | 8 |
| 53 | 11.8275933700664 | high | 8 |
| 54 | 10.985788480584 | high | 8 |
| 55 | 11.3298206853252 | high | 8 |
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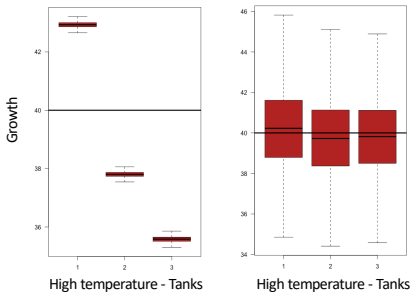
| 58 | 10.6686619304702 | high | 9 |
| 59 | 12.050356325582 | high | 9 |
| 60 | 11.352528167564 | high | 9 |
| 61 | 12.774538937538 | high | 9 |
| 62 | 8.7132041741008 | high | 9 |
| 63 | 10.9823492737741 | high | 9 |

8

## The plural of anecdote is not data (Roger Brinner)

**Case 1**
(random effect very strong, i.e., more uncertainty/variation among replicates (tanks))

**Case 2**
(random effect weak, i.e., small uncertainty/variation among replicates (tanks))







9

## Mixed models for ANOVAs (tutorial 9)

Sources of variation:

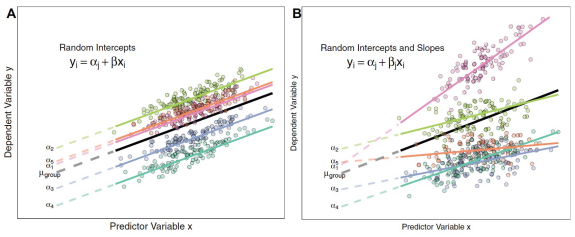
**Fixed effect model -**
Effects of treatments (e.g., temperature)
Residuals

**Mixed effect model (fixed + random effect) -**
Effects of treatments (e.g., temperature)
Residuals
Variation among replicates within fixed effect (e.g., tank)

10

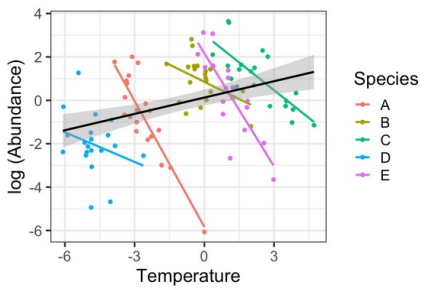
## Understanding mixed models for regressions via a two-stage method!

11




From Harrison et al. (2018) PeerJ 6:e4794

12




13

Zuur et al. (2007) used marine benthic data from **nine inter-tidal areas** along the Dutch coast collected by the RIKZ institute (summer of 2002).

In **each intertidal zone** (zone where ocean meets land; denoted by 'beach'), five samples were taken, and the macro-fauna and abiotic variables were measured.

The goal is to model how species richness change as a function of **NAP** (Normal Amsterdam Level: the height of a sampling station compared to mean tidal level) and **Exposure** - a nominal index for the entire beach (high/low) composed of the following elements: wave action, length of the surf zone, slope, grain size, and the depth of the anaerobic layer.

$$R_{ij} = b_0 + b_1 \times NAP_{ij} + b_2 \times Exposure_j + e_{ij}$$

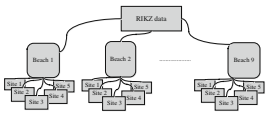
Each site for each beach has a NAP value

One value per beach

$$\varepsilon_{ij} \sim N(0, \sigma^2)$$

$i$ = sites;
$j$ = beach

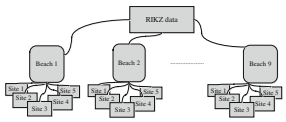
Zuur AF, Ieno EN, Smith GM (2007) Analysing Ecological Data. Springer.




14

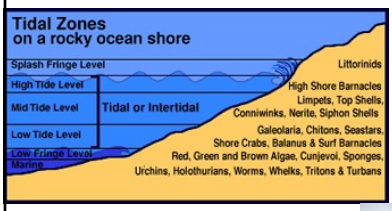
RIKZ data

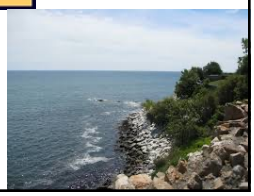
| Sample | Richness | Exposure | NAP | Beach |
|---|---|---|---|---|
| 1 | 11 | 1 | 0.045 | 1 |
| 2 | 10 | 1 | -1.036 | 1 |
| 3 | 13 | 1 | -1.336 | 1 |
| 4 | 11 | 1 | 0.616 | 1 |
| 5 | 10 | 1 | -0.684 | 1 |
| 6 | 8 | 1 | 1.19 | 2 |
| 7 | 9 | 1 | 0.82 | 2 |
| 8 | 8 | 1 | 0.635 | 2 |
| 9 | 19 | 1 | 0.061 | 2 |
| 10 | 17 | 1 | -1.334 | 2 |
| 11 | 6 | 2 | -0.976 | 3 |
| 12 | 1 | 2 | 1.494 | 3 |
| 13 | 4 | 2 | -0.201 | 3 |
| 14 | 3 | 2 | -0.482 | 3 |
| 15 | 3 | 2 | 0.167 | 3 |
| 16 | 1 | 2 | 1.768 | 4 |
| 17 | 3 | 2 | -0.03 | 4 |
| 18 | 3 | 2 | 0.46 | 4 |
| 19 | 1 | 2 | 1.367 | 4 |
| 20 | 4 | 2 | -0.811 | 4 |
| 21 | 3 | 1 | 1.117 | 5 |
| 22 | 22 | 1 | -0.503 | 5 |
| 23 | 6 | 1 | 0.729 | 5 |
| . | | | | |
| . | | | | |
| . | | | | |
| 45 | | | | |




15

$$R_{ij} = b_0 + b_1 \times NAP_{ij} + e_{ij}$$



16

**Understanding mixed models for regressions via a two-stage method!**

Mixed effects models for regression are often introduced first by using an easy-to-understand framework called two-stage analysis.

We then understand better how a mixed model for regression works BUT also understand that the two-stage analysis is not optimal for the analysis.

Then the two-stages (or multiple stages) of the model are combined into a single mixed effect model.

17

Understanding mixed models via a two-stage method!

The first stage is to fit a linear regression model to each category of the random factor (here beach). Separate intercepts and slopes are calculated for each beach.

$$R_{i1} = b_0 + b_1 \times NAP_{i1} + e_i \qquad j = 1$$

$$R_{i2} = b_0 + b_1 \times NAP_{i2} + e_i \qquad j = 2$$

$$\ldots\ldots.$$

$$R_{i9} = b_0 + b_1 \times NAP_{i9} + e_i \qquad j = 9$$

Each beach would have a different slope and intercept

18

## Understanding mixed models via a two-stage method!

The first stage is to fit a linear regression model to each category of the random factor (here beach). Separate intercepts and slopes are calculated for each beach. HERE BEACH 1 WAS MODELLED

$$R_{i1} = b_0 + b_1 \times NAP_{i1} + e_i$$

$$\begin{pmatrix} R_{11} \\ R_{21} \\ R_{31} \\ R_{41} \\ R_{51} \end{pmatrix} = \begin{pmatrix} 1 & NAP_{11} \\ 1 & NAP_{21} \\ 1 & NAP_{31} \\ 1 & NAP_{41} \\ 1 & NAP_{51} \end{pmatrix} \times \begin{pmatrix} b_{0_1} \\ b_{11} \end{pmatrix} + \begin{pmatrix} e_1 \\ e_2 \\ e_3 \\ e_4 \\ e_5 \end{pmatrix}$$

$i$ = sites;
$j$ = beach

**R***i* is a vector of length 5 containing the species richness values of the 5 sites on beach 1

19

## Understanding mixed models via a two-stage method!

The first stage is to fit a linear regression model to each category of the random factor (here beach). Separate intercepts and slopes are calculated for each beach.

$$R_{i1} = b_0 + b_1 \times NAP_{i1} + e_i$$

Let's say beach 1 had 4 observations instead of 5, then:

$$\begin{pmatrix} R_{11} \\ R_{21} \\ R_{31} \\ R_{41} \end{pmatrix} = \begin{pmatrix} 1 & NAP_{11} \\ 1 & NAP_{21} \\ 1 & NAP_{31} \\ 1 & NAP_{41} \end{pmatrix} \times \begin{pmatrix} b_{0_1} \\ b_{1_1} \end{pmatrix} + \begin{pmatrix} e_1 \\ e_2 \\ e_3 \\ e_4 \end{pmatrix}$$

20

## RIKZ data

| | A | B | C | D | E |
|---|---|---|---|---|---|
| | Sample | Richness | Exposure | NAP | Beach |
| | 1 | 11 | 1 | 0.045 | 1 |
| | 2 | 10 | 1 | -1.036 | 1 |
| | 3 | 13 | 1 | -1.336 | 1 |
| | 4 | 11 | 1 | 0.616 | 1 |
| | 5 | 10 | 1 | -0.684 | 1 |
| | 6 | 8 | 1 | 1.19 | 2 |
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| | 9 | 19 | 1 | 0.061 | 2 |
| | 10 | 17 | 1 | -1.334 | 2 |
| | 11 | 6 | 2 | -0.976 | 3 |
| | 12 | 1 | 2 | 1.494 | 3 |
| | 13 | 4 | 2 | -0.201 | 3 |
| | 14 | 3 | 2 | -0.482 | 3 |
| | 15 | 3 | 2 | 0.167 | 3 |
| | 16 | 1 | 2 | 1.768 | 4 |
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| | 22 | 22 | 1 | -0.503 | 5 |
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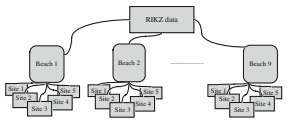
| | . | | | | |
| | . | | | | |
| | . | | | | |
| | 45 | | | | |




21

## Understanding mixed models via a two-stage method!

The first stage is to fit a linear regression model to each category of the random factor (here beach). Separate intercepts and slopes are calculated for each beach.

$$R_{ij} = b_0 + b_1 \times NAP_{ij} + e_{ij} \qquad j = 1, \ldots, 4$$

```
2
3  RIKZ <- read.table("RIKZ.txt",header=TRUE)
4  Beta <- vector()
5  for (i in 1:9){
6    result <- summary(lm(Richness ~ NAP,subset = (Beach==i), data=RIKZ))
7    Beta[i] <- result$coefficients[2, 1]
8  }
9
```

22

## Understanding mixed models via a two-stage method!

The first stage is to fit a linear regression model to each category of the random factor (here beach). Separate intercepts and slopes are calculated for each beach.

$$R_{ij} = b_0 + b_1 \times NAP_{ij} + e_{ij} \qquad j = 1, \ldots, 4$$

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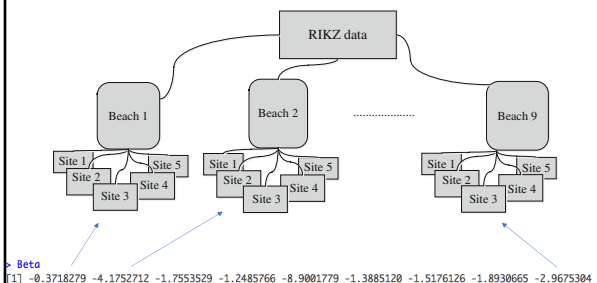
7    Beta[i] <- result$coefficients[2, 1]
8  }
9
```

```
> Beta
[1] -0.3718279 -4.1752712 -1.7553529 -1.2485766 -8.9001779 -1.3885120 -1.5176126 -1.8930665 -2.9675304
```

Lots of differences in slopes among beaches!

23

## RIKZ data

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> Beta
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```

24

## Understanding mixed models via a two-stage method!

The first stage is to fit a linear regression model to each category of the random factor (here beach). Separate intercepts and slopes are calculated for each beach.

$$R_{i1} = b_0 + b_1 \times NAP_{i1} + e_i \qquad j = 1$$

$$R_{i2} = b_0 + b_1 \times NAP_{i2} + e_i \qquad j = 2$$

$$\ldots\ldots$$

$$R_{i9} = b_0 + b_1 \times NAP_{i9} + e_i \qquad j = 9$$

Each beach would have a different slope and intercept

Remember that *i* represents the sites within each beach

25

## The second step fits the estimated regression slopes as a function of exposure. Given that expose is a nominal variable, this would just a simple one-way ANOVA:

slope of Exposure for the slopes of R on NAP

Residuals for the slopes

$$\hat{\beta}_j = \eta + \tau \times Exposure_j + e_{b_j} \qquad j = 1, \ldots, 9$$

Intercept

```
> Beta
[1] -0.3718279 -4.1752712 -1.7553529 -1.2485766 -8.9001779 -1.3885120 -1.5176126 -1.8930665 -2.9675304
```

$j$ = beach

How does the influence of NAP on richness (slopes of R on NAP) change as a function of exposure?

26

## The second step fits the estimated regression slopes as a function of exposure. Given that expose is a nominal variable, this would just a simple one-way ANOVA:

slope of Exposure for the slopes of R on NAP

Residuals for the slopes

$$\hat{\beta}_{i_{e_{b_i}}} = \eta + \tau \times Exposure_{e_{b_i}} + e_{b_{i_{e_{b_i}}}} \qquad e_{b_i} = 1, \ldots, 9$$

Intercept

```
> Expose <- factor(c(0, 0, 1, 1, 0, 1, 1, 0, 0))
> anova(lm(Beta ~ Expose))
Analysis of Variance Table

Response: Beta
          Df Sum Sq Mean Sq F value Pr(>F)
Expose     1 10.600 10.6003  1.7551 0.2268
Residuals  7 42.278  6.0397
```

No significant effect of exposure on the individual beach slopes

27

The second step fits the estimated regression slopes as a function of exposure. Given that expose is a nominal variable, this would just a simple one-way ANOVA:

$$\widehat{\boldsymbol{\beta}_j} = \mathbf{K}_i \times \gamma + e_{b_j} \quad e_{b_j} \sim N(0, D)$$

$$\begin{pmatrix} -0.37 \\ -4.17 \\ -1.75 \\ -1.24 \\ -8.90 \\ -1.38 \\ -1.51 \\ -1.89 \\ -2.96 \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 1 & 0 \\ 1 & 1 \\ 1 & 1 \\ 1 & 0 \\ 1 & 1 \\ 1 & 1 \\ 1 & 0 \\ 1 & 0 \end{pmatrix} \times \begin{pmatrix} \eta \\ \tau \end{pmatrix} + \begin{pmatrix} e_{b_1} \\ e_{b_2} \\ e_{b_3} \\ e_{b_4} \\ e_{b_5} \\ e_{b_6} \\ e_{b_7} \\ e_{b_8} \\ e_{b_9} \end{pmatrix}$$

28

Understanding mixed models via a two-stage method!

The two formulae of the two-stage approach (more predictors, more stages) and some issues:

$$\mathbf{R}_i = \mathbf{Z}_i \times b_i + e_i \quad e_i \sim N(0, \sigma^2)$$
$$\widehat{\boldsymbol{\beta}_j} = \mathbf{K}_j \times \gamma + e_{b_j} \quad e_{b_j} \sim N(0, D)$$

hyperparameter (assumed independent)

1) all the data from a beach is summarized by one parameter (intercept and slope per beach).

2) We analyzed regression parameters, not the observed data; i.e., the variable of interest is not modelled directly but rather the slopes or intercepts or both.

3) The number of observations used to calculate the summary statistic (slopes) is not used in the second step. In this case, we had five observations for each beach. But if you have 5, 50, or 50,000 observations, you still end up with only one summary statistic.

Zuur AF, Ieno EN, Smith GM (2007) Analysing Ecological Data. Springer.

29

The more appropriate procedure:
Mixed models in one-single step

(next lecture)

30