Two stage design

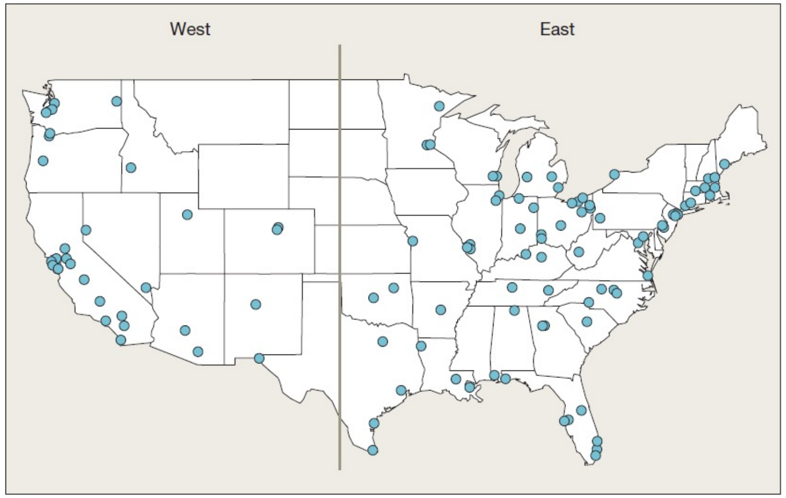
Aurelio Tobias

Spanish Research Council (CSIC)

Outline

- Introduction
- Time-series designs
- Two stage design
- Univariate meta-analysis
- Multivariate meta-analysis

Multisite time-series studies



Measurements are for 108 counties. The vertical line divides the east and west regions. PM_{10} indicates particulate matter is 10 μm or less in aerodynamic diameter; $PM_{2.5}$, particulate matter is 2.5 μm or less in aerodynamic diameter.

Peng et al. JAMA 2008

- How to summarize the results?
- How to estimate the global association?
- How to explain the local associations?

Time-series regression designs

• **Summarized** design – Aggregates data for all cities in a single time-series data $$Y_t = s(t) + cb(\bar{x}_{t,l})$$	Straightforward approach is simple to carry out Doesn't allow for exploring heterogeneity Difficult to understand the representativeness of the exposure at the country level
• **Pooled** design – Combines data for all cities in a unique data set $$y_{ti} = s(t_i) + cb\left(x_{ti,l}\right) + \beta city_i$$	Marginal approach still easy to analyze It can be made conditional fitting complex models to derive city-specific and explore heterogeneity Assumes the same association in each city
• **Two-stage** design – Estimates city specific associations combined (stage 1) to combine them afterwards (step 2) $$y_{t\|i} = s(t) + cb\left(x_{t,l}\right) \;\; for\ cities\ i = 1{:}\,k$$ $$\theta_i \sim N(X_i\beta, S_i + \Sigma)$$	Flexible approach modeling city-specific associations and allows explaining heterogeneity Comparing/combining city-specific estimates may be difficult because of different reference values Modelling cities with small counts

Two stage design

- **First stage**
- Modelling location-specific time-series analysis

- **Second stage**
- Meta-analysis technique to derive global (overall) summary measures pooling the first stage location-specific effect estimates
- Meta-regression models to explain the variability between location-specific effect estimates

First-stage estimates

$$y_t^s \sim Poisson\ (\lambda_t^s)$$

$$log(\lambda_t^s) = \alpha_0^s + \beta^s x_t^s + \text{other factors}_t^s$$

- β^s is the effect estimate for the exposure of interest (**log-relative risk**) for each location (s = 1, …, S)
  - exp(β) = Relative Risk (RR) per a unit increase of the exposure on a single day
- Normal approximation $\hat{\beta}^s | \beta^s \sim N(\beta^s, \nu^s)$
  - $\hat{\beta}^s$ is the estimated effect of the exposure
  - β^s is the true effect of the exposure
  - ν^s is the within-location variance of the true effect, which depends on the number of days (sample size) and the predictive power of the 1st stage models

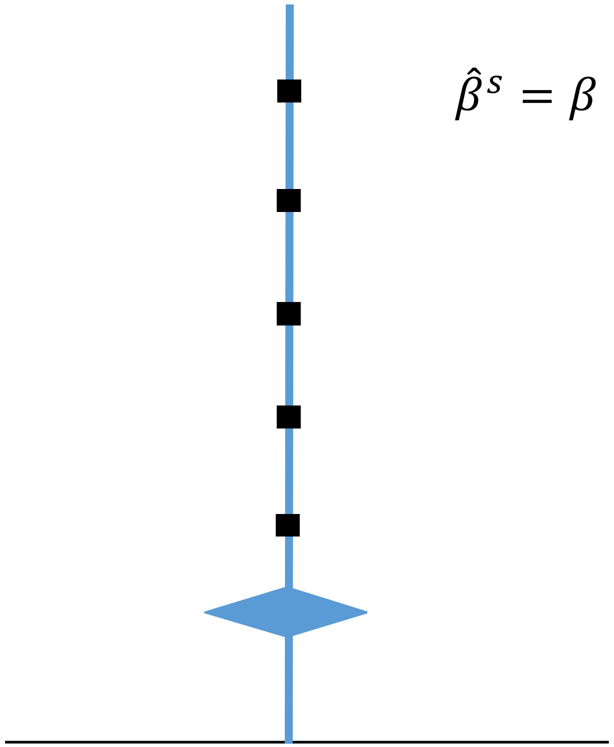
Second-stage univariate meta-analysis

- Pooling non-linear exposure-response curve pooled across multiple locations
- Univariate *vs.* multivariate meta-analysis
- Single *vs.* multiple coefficients for the association

- **Steps in practice**
- First-stage modelling with `crossbasis` and `glm` functions
- Reduce the number of parameters using `crossreduce` function
- Second-stage modelling with `rma` or `mixmeta` functions

Second-stage univariate meta-analysis

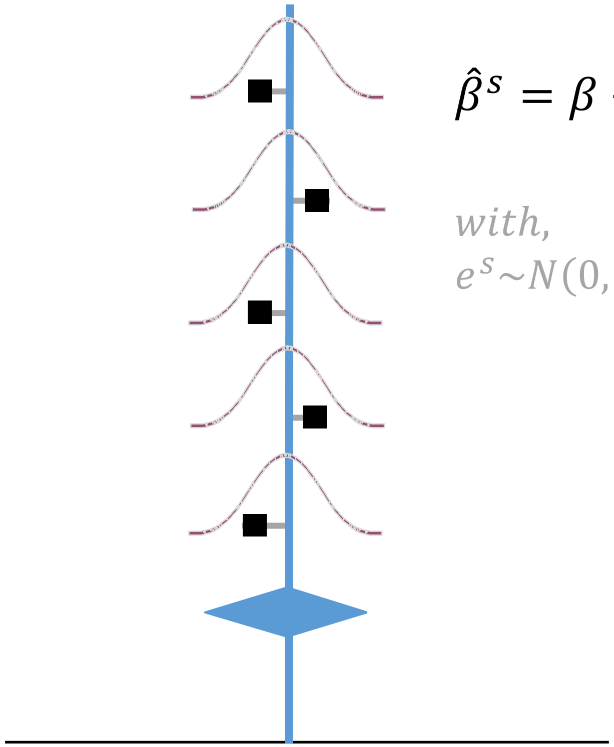
- Fixed effects model



$$\hat{\beta}^s = \beta$$

Second-stage univariate meta-analysis

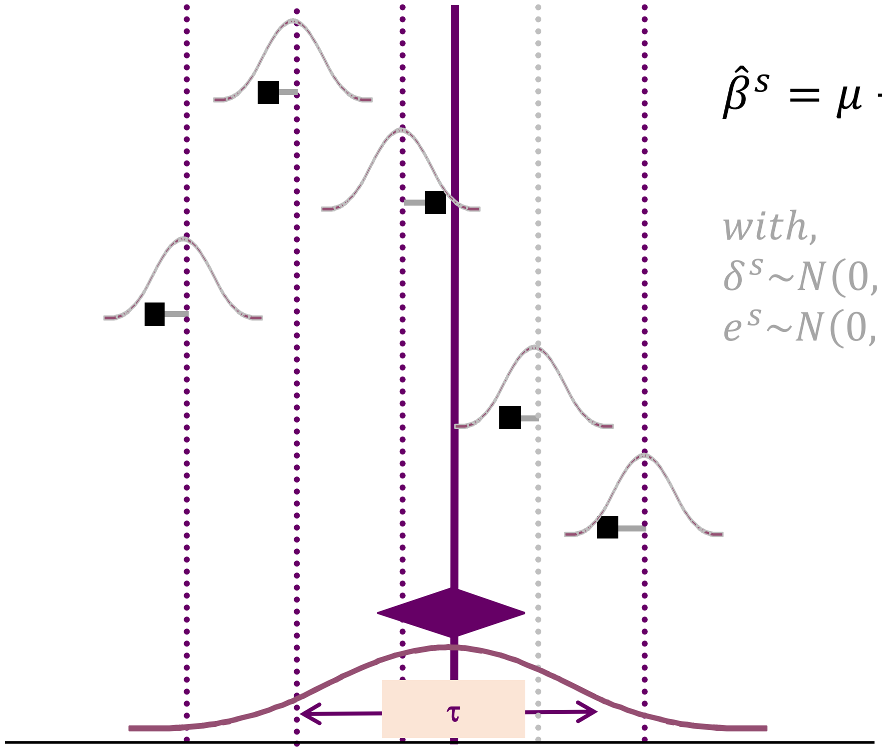
- Fixed effects model



$$\hat{\beta}^s = \beta + e^s$$

with,
$e^s \sim N(0, \upsilon^s)$

- Random effects model



$$\hat{\beta}^s = \mu + \delta^s + e^s$$

with,
$\delta^s \sim N(0, \tau)$
$e^s \sim N(0, \upsilon^s)$

Second-stage univariate meta-analysis

- **Fixed effects model**
- Assumes homogeneity of effects
- Meta-analysis pooled estimate

$$\hat{\beta} = \sum w^s \hat{\beta}^s \Big/ \sum w^s$$

$$with\ variance\ V(\hat{\beta}) = 1 \Big/ \sum w^s$$

- $weigths\ w^s = 1/v$

- **Random effects model**
- Assumes that the effects are randomly distributed with fixed mean and variance
- Meta-analysis pooled estimate

$$\hat{\mu} = \sum w_*^s \hat{\beta}^s \Big/ \sum w_*^s$$

$$with\ variance\ V(\hat{\mu}) = 1 \Big/ \sum w_*^s$$

- $weigths\ w_*^s = 1/(v + \tau)$

Second-stage meta-analysis

- **Testing for heterogeneity**
- Assumes homogeneity of effects

$$Q = \sum w^s(\hat{\beta}^s - \hat{\beta}) \sim \chi^2_{n-1}$$

- The test has low power when there are few locations, but high when there are many. Thus, the p-value is difficult to interpret

- **Quantifying heterogeneity**
- Based on Q

$$I^2 = \frac{Q - (n-1)}{Q}$$

- Proportion of total variability explained by heterogeneity
- Cut-off values of 25%, 50% and 75% might be considered as low, moderate, high and very high heterogeneity, respectively

First-stage modelling in R

- Daily time-series, between 1993-2006, in 10 regions in the UK

```
> dlist <-split(data, data$regnames)
> summary(dlist)
          Length Class      Mode
E-Mid     15     data.frame list
East      15     data.frame list
London    15     data.frame list
N-East    15     data.frame list
N-West    15     data.frame list
S-East    15     data.frame list
S-West    15     data.frame list
W-Mid     15     data.frame list
Wales     15     data.frame list
York&Hum  15     data.frame list
```

```
> head(select(data, region, regnames, date, all, tmean))
  region regnames       date  all      tmean
1      1   N-East 1993-01-01  125  2.6037474
2      1   N-East 1993-01-02   96  0.1191766
3      1   N-East 1993-01-03   92 -2.9129980
4      1   N-East 1993-01-04   80  1.6405052
5      1   N-East 1993-01-05  103  4.3069959
6      1   N-East 1993-01-06  119  4.4676647

> tail(select(data, region, regnames, date, all, tmean))
      region regnames       date  all    tmean
51125     10    Wales 2006-12-26   87 3.688205
51126     10    Wales 2006-12-27   82 6.161925
51127     10    Wales 2006-12-28  100 7.684133
51128     10    Wales 2006-12-29  121 9.484643
51129     10    Wales 2006-12-30  107 8.651460
51130     10    Wales 2006-12-31   82 9.130294
```

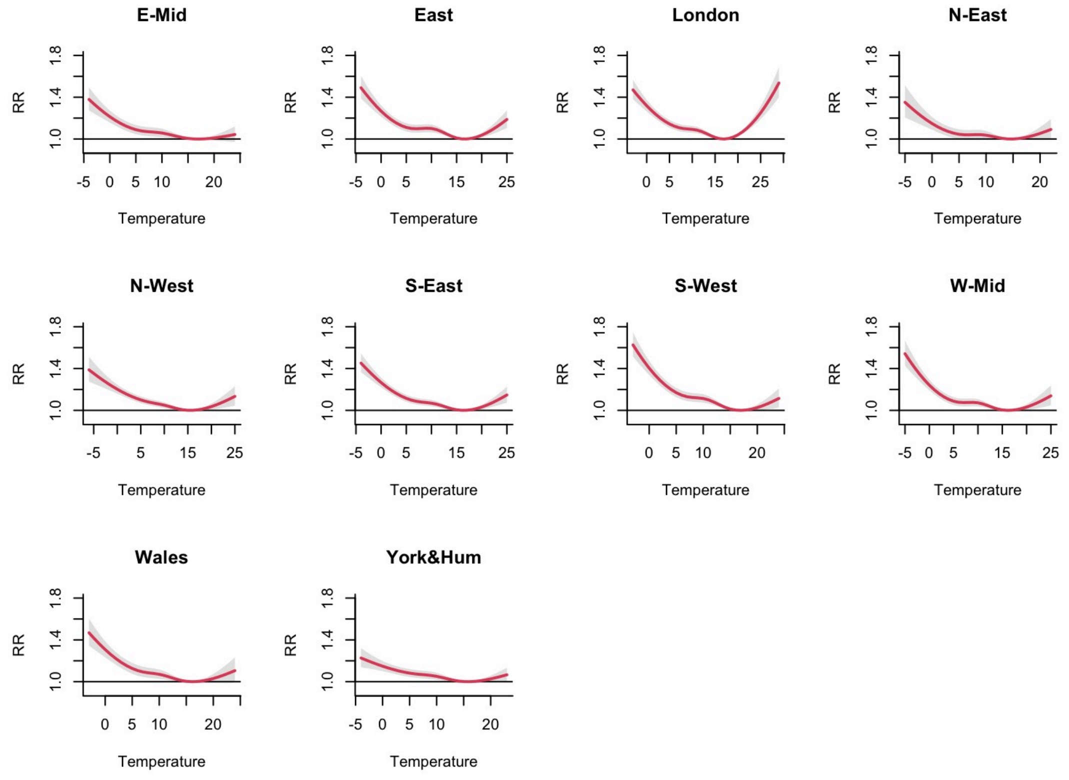
- Non-linear association between temperature and mortality
- Focus on the heat effect (99^{th} percentile *vs.* MMT)

First-stage modelling in R

```
# Vector to store risk estimates for univariate meta-analysis.
regions <- names(dlist)
logRR <- logRRse <- vector("numeric",10)

# Matrix to store reduced crossbasis paremeters for multivariate meta-
analysis.
coef <- matrix(NA, nrow=length(regions), ncol=3+1,
               dimnames=list(regions, paste0("b",seq(4))))
vcov <- vector("list", length(regions))

# Figure for region specific exposure-response curves.
par(mex=0.8, mfrow=c(3,4))
```

```
# Loop for region specific analysis.
for(i in seq(regions)){
  cat(i,"")
  sub <- dlist[[i]]
  pct <- quantile(sub$tmean,prob=c(.01,.10,.25,.50,.75,.90,.99), na.rm=T)
  varknot <- pct[c(3:5)]
  cb.temp <- crossbasis(sub$tmean, lag=14,
                        argvar=list(fun="ns", knots=varknot),
                        arglag=list(fun="ns", knots=c(2,5)))
  model <- glm(all ~ cb.temp + ns(time, df = 10*14) + dow, sub,
               family=quasipoisson)
  pred.temp <- crosspred(cb.temp, model, by=1)

  # Predicted exposure-response curve centered at the MMT.
  cen <- findmin(cb.temp, model)
  pred.heat <- crosspred(cb.temp, model, cen=cen, by=1)

  # Plot region specific exposure-response curve.
  plot(pred.heat, "overall", ylim=c(0.9,1.8), col=2, lwd=2,
       xlab="Temperature", ylab="RR", main=regions[i])

  # Store risk estimates for univariate meta-analysis.
  target     <- as.character(round(pct[7]))
  logRR[i]   <- pred.heat$allfit[target]
  logRRse[i] <- pred.heat$allse[target]

  # Store reduced crossbasis parameters for multivariate meta-analysis.
  cr         <- crossreduce(cb.temp, model)
  coef[i,]   <- coef(cr)
  vcov[[i]]  <- vcov(cr)
}

# End of figure.
layout(1)
```

First-stage modelling in R



- Risk estimates for univariate meta-analysis

```
> cbind(logRR, logRRse)

            logRR    logRRse
 [1,] 0.01683265 0.01773714
 [2,] 0.08284556 0.01959752
 [3,] 0.17801107 0.02053977
 [4,] 0.05228360 0.02809548
 [5,] 0.05061887 0.01881418
 [6,] 0.06825094 0.01842209
 [7,] 0.04334580 0.02035968
 [8,] 0.04694394 0.01840349
 [9,] 0.02975509 0.02089360
[10,] 0.03782420 0.02031425
```

Second-stage univariate meta-analysis in R

```
# Random effects meta-analysis.
uni <- rma(y=logRR, sei=logRRse, slab=regions, measure="RR")
summary(uni)
ci.exp(uni)
```

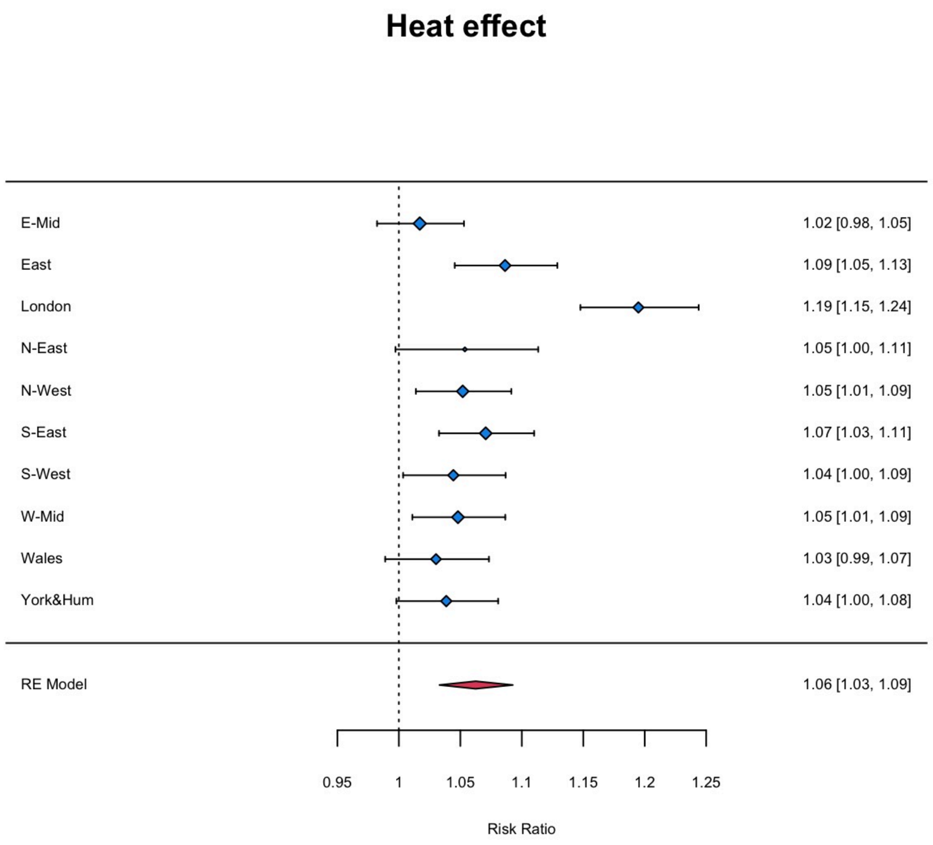
```
# Forest plot.
forest(uni, transf=exp, refline=1, pch=23, bg=4, col=2,
       main="Heat effect")
```

```
> summary(uni)
Random-Effects Model (k = 10; tau^2 estimator: REML)

  logLik  deviance       AIC       BIC      AICc
 15.0841  -30.1683  -26.1683  -25.7738  -24.1683

tau^2 (estimated amount of total heterogeneity): 0.0016 (SE =
0.0010)
tau (square root of estimated tau^2 value):      0.0405
I^2 (total heterogeneity / total variability):   80.48%
H^2 (total variability / sampling variability):  5.12

Test for Heterogeneity:
Q(df = 9) = 45.2714, p-val < .0001

Model Results:

estimate      se    zval    pval   ci.lb   ci.ub
  0.0605  0.0143  4.2265  <.0001  0.0325  0.0886  ***

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> ci.exp(uni)
        exp(Est.)     2.5%    97.5%
intrcpt  1.062415 1.033001 1.092666
```

Second-stage univariate meta-analysis in R

```
# Random effects meta-analysis.
uni <- rma(y=logRR, sei=logRRse, slab=regions, measure="RR")
summary(uni)
ci.exp(uni)
```

```
# Forest plot.
forest(uni, transf=exp, refline=1, pch=23, bg=4, col=2,
       main="Heat effects")
```



Second-stage meta-regression

- Weighted random effects linear regression model

$$\beta^s = \mu + \sum \alpha_j W_j^s + \delta^s + e^s$$

with,

$$\beta^s | \mu, \alpha_1, \dots, \alpha_p, \sigma^2 \sim N(\mu + \sum_{j=1}^{p} \alpha_j W_j^s, \sigma^2)$$

- β^s is the outcome variable, as the location-specific **log-relative risk**
- W_j^s are explanatory variables (meta-predictors) that **characterize the locations** s
- μ is the intercept, as the **overall association** between exposure and outcome when the meta-predictors W_j^s are centred as $(W_j^s - \overline{W}_j)$
- α_j are the regression parameters, indicating the **change** in the **log-relative risk** associated for **a unit change** in the location-specific **meta-predictors** W_j^s
- σ^2 is the between-location variance, indicating **heterogeneity** of the log-relative risks across locations that could be explained by the meta-predictors W_j^s

Second-stage meta-regression in R

```
# Generate Latitude data.
lat <- c(54.84815, 53.58832, 53.72352, 52.85539, 52.53304,
         52.03734, 51.50583, 51.24213, 51.05361, 52.02615)
```

```
# Meta-regression by latitude.
res <- rma(y=logRR, sei=logRRse, mods=lat)
summary(res)
```

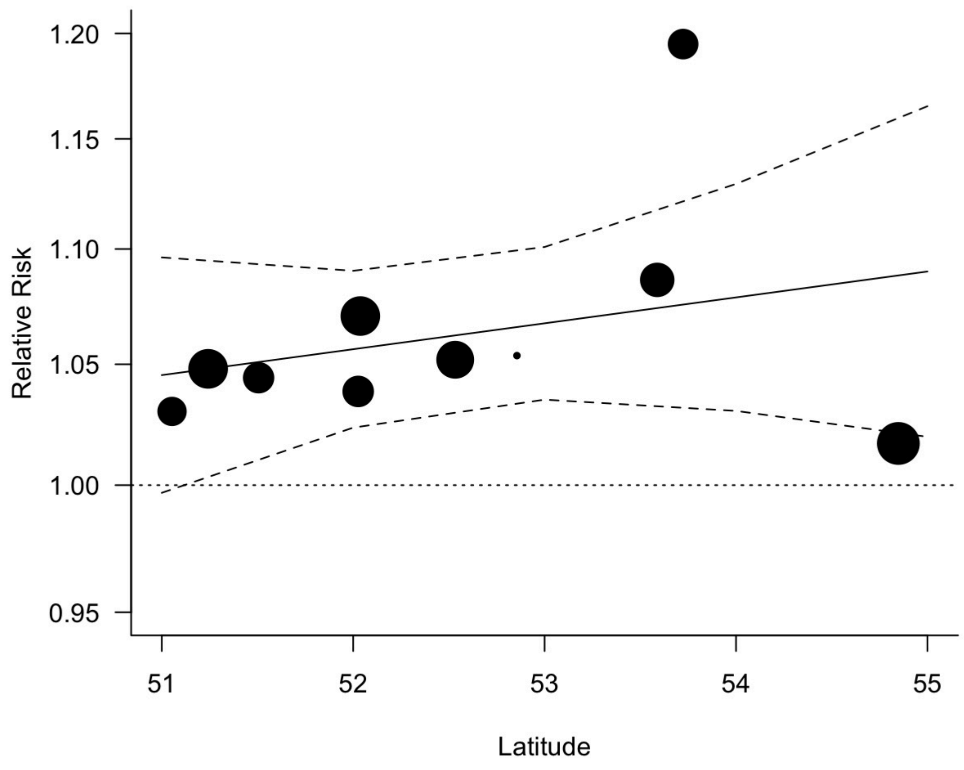
```
# Bubble plot.
preds <- predict(res, newmods = cbind(51:55), transf = exp)
wi <- 1/sqrt(logRRse)
size <- 0.5 + 3 * (wi - min(wi))/(max(wi) - min(wi))

plot(lat, exp(logRR), xlim=c(51,55), ylim=c(0.95,1.2), pch=19,
     cex=size, xlab="Latitude", ylab="Relative Risk", las=1,
bty="l", log="y")
lines(51:55, preds$pred)
lines(51:55, preds$ci.lb, lty="dashed")
lines(51:55, preds$ci.ub, lty="dashed")
abline(h=1, lty = "dotted")
```

```
> summary(res)

Mixed-Effects Model (k = 10; tau^2 estimator: REML)

  logLik  deviance       AIC       BIC      AICc
 13.2646  -26.5292  -20.5292  -20.2909  -14.5292

tau^2 (estimated amount of residual heterogeneity):     0.0017
(SE = 0.0011)
tau (square root of estimated tau^2 value):             0.0416
I^2 (residual heterogeneity / unaccounted variability): 81.08%
H^2 (unaccounted variability / sampling variability):   5.29
R^2 (amount of heterogeneity accounted for):            0.00%

Test for Residual Heterogeneity:
QE(df = 8) = 43.1020, p-val < .0001

Test of Moderators (coefficient 2):
QM(df = 1) = 0.6985, p-val = 0.4033

Model Results:

         estimate      se     zval    pval    ci.lb   ci.ub
intrcpt   -0.4888  0.6574  -0.7435  0.4572  -1.7773  0.7998
mods       0.0105  0.0125   0.8358  0.4033  -0.0141  0.0350

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Second-stage meta-regression in R

```
# Generate Latitude data.
lat <- c(54.84815, 53.58832, 53.72352, 52.85539, 52.53304,
         52.03734, 51.50583, 51.24213, 51.05361, 52.02615)

# Meta-regression by latitude.
res <- rma(y=logRR, sei=logRRse, mods=lat)
summary(res)
```

```
# Bubble plot.
preds <- predict(res, newmods = cbind(51:55), transf = exp)
wi <- 1/sqrt(logRRse)
size <- 0.5 + 3 * (wi - min(wi))/(max(wi) - min(wi))

plot(lat, exp(logRR), xlim=c(51,55), ylim=c(0.95,1.2), pch=19,
     cex=size, xlab="Latitude", ylab="Relative Risk", las=1,
bty="l", log="y")
lines(51:55, preds$pred)
lines(51:55, preds$ci.lb, lty="dashed")
lines(51:55, preds$ci.ub, lty="dashed")
abline(h=1, lty = "dotted")
```



Limitations

- The standard two-stage design requires that the association of interest can be represented by a **single parameter**
- This is key limitation for assessing **complex relationships** (e.g., non-linear associations)
- Possible solutions
    - Simplify the relationship assuming **linear or linear-threshold** shapes
    - Summarize the relationship looking at **specific comparisons** (e.g., percentiles)
    - Restrict the analysis looking at **periods where linearity holds** (e.g., summer for heat)
- But all the methods above can introduce **biases**
- The second-stage modelling can be extended to multivariate **meta-analysis**

Second-stage multivariate meta-analysis

- Linear association

$y_t^s \sim Poisson\ (\lambda_t^s)$

$log(\lambda_t^s) = \alpha_0^s + \beta^s x_t^s + \text{other factors}_t^s$

- Non-linear association

$y_t^s \sim Poisson\ (\lambda_t^s)$

$log(\lambda_t^s) = \alpha_0^s + ns(x_t^s, df) + \text{other factors}_t^s$

- Univariate meta-analysis

$\beta^s = (\hat{\beta}^s)$

$v^s = (\hat{v}^s) + (\tau\ \ )$

- Multivariate meta-analysis

$$\beta^s = \begin{pmatrix} \hat{\beta}_1^s \\ \hat{\beta}_2^s \end{pmatrix}$$

$$V^s = \begin{pmatrix} \hat{v}_1^s & \hat{v}_{12}^s \\ \hat{v}_{12}^s & \hat{v}_2^s \end{pmatrix} + \begin{pmatrix} \tau_1 & \tau_{12} \\ \tau_{12} & \tau_2 \end{pmatrix}$$

Second-stage multivariate meta-analysis

- It can be extended to multivariate meta-regression

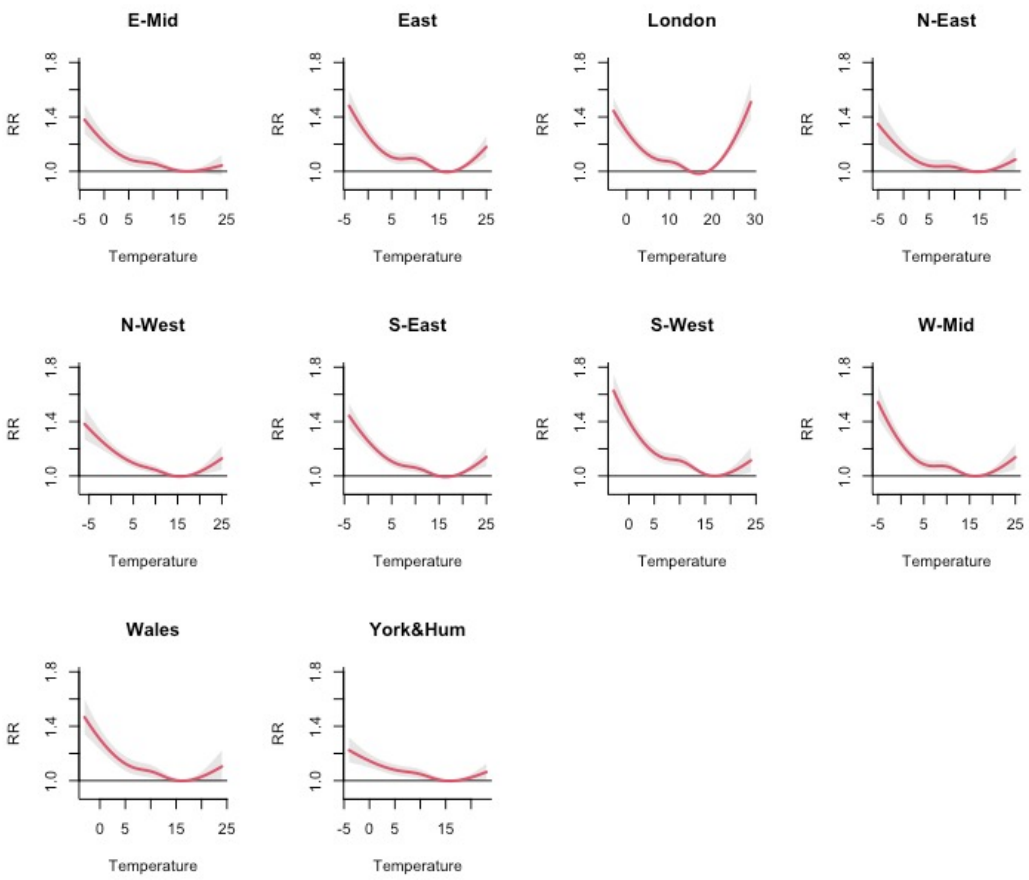
$$\beta = W\alpha + Z\delta + e$$

$with,$
$\alpha \sim N(0, \Psi)$
$\delta \sim N(0, S)$

$and,$
$\beta \sim N(W\alpha, \Sigma)$
$\Sigma = S + Z\Psi Z$

- $W\alpha$ defines the **fixed-effects** that represent the population averaged outcomes in terms of explanatory variables (meta-predictors W) with fixed effects coefficients α
- $Z\delta$ defines the **random-effects** as the deviation from the population averages at different grouping levels composing the random effects matrix design Z with effects coefficients δ
- Σ is the **variance-covariance matrix** of within-group errors (S) and between-group random effects (Ψ)

First stage modelling in R



- Reduced crossbasis parameters for multivariate meta-analysis

```
> summary(model)
...
Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)       5.4333188  0.0736495  73.773  < 2e-16 ***
cb.tempv1.l1     -0.0487452  0.0109659  -4.445 8.97e-06 ***
cb.tempv1.l2     -0.0014946  0.0087759  -0.170 0.864778
cb.tempv1.l3      0.0114526  0.0114114   1.004 0.315614
cb.tempv1.l4     -0.0230942  0.0087345  -2.644 0.008219 **
cb.tempv2.l1     -0.0585628  0.0104021  -5.630 1.90e-08 ***
cb.tempv2.l2      0.0004696  0.0087611   0.054 0.957251
cb.tempv2.l3      0.0049325  0.0108768   0.453 0.650215
cb.tempv2.l4     -0.0163957  0.0084476  -1.941 0.052329 .
cb.tempv3.l1     -0.1563530  0.0257112  -6.081 1.28e-09 ***
cb.tempv3.l2     -0.0066095  0.0209955  -0.315 0.752923
cb.tempv3.l3      0.0777845  0.0269277   2.889 0.003886 **
cb.tempv3.l4     -0.0804452  0.0206833  -3.889 0.000102 ***
cb.tempv4.l1     -0.1125320  0.0119571  -9.411  < 2e-16 ***
cb.tempv4.l2     -0.0162316  0.0098028  -1.656 0.097823 .
cb.tempv4.l3      0.1096685  0.0122032   8.987  < 2e-16 ***
cb.tempv4.l4     -0.0932488  0.0094892  -9.827  < 2e-16 ***
...

> coef(cr)
         b1          b2          b3          b4
-0.15183316 -0.19649052 -0.31282933 -0.07183865
```

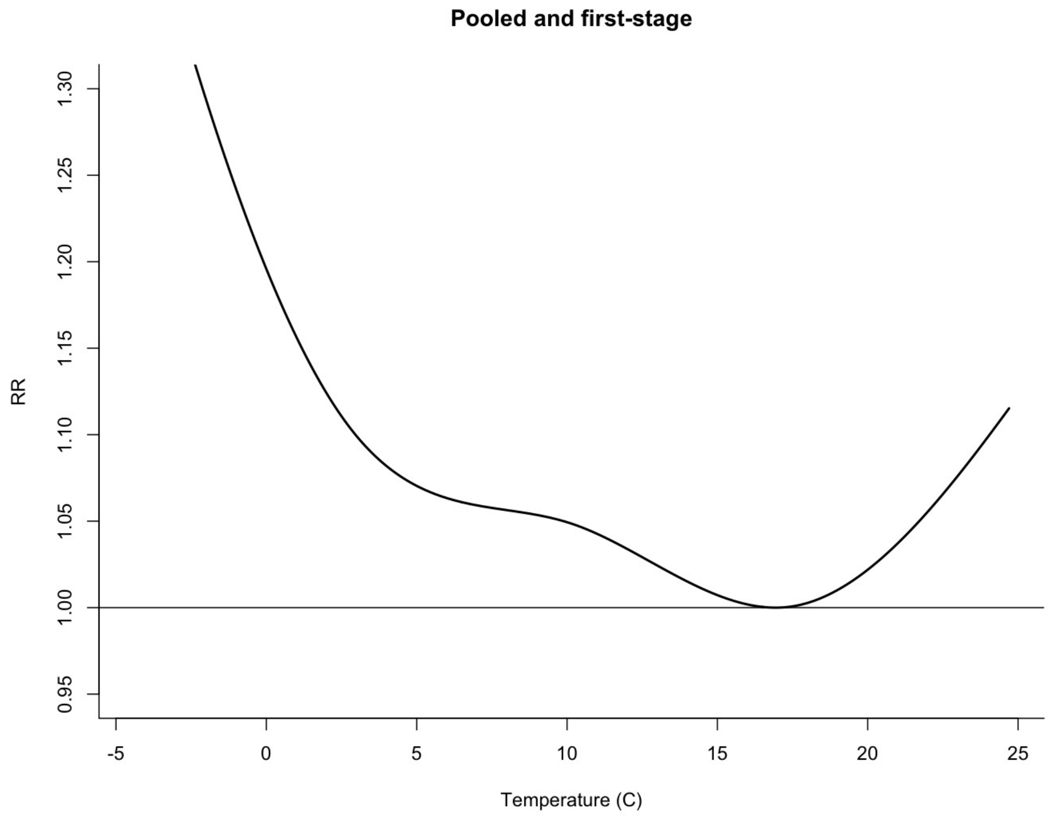
Second stage multivariate meta-analysis in R

```
# Multivariate mixed-effects meta-analysis.
mv <- mixmeta(coef~1, vcov, method="ml")
summary(mv)

# Generate the temperature distribution for prediction.
bound <- rowMeans(sapply(dlist, function(x) range(x$tmean)))
xvar <- seq(bound[1], bound[2], by=0.1)

# Predicted exposure-response curve from meta-analysis estimates.
argvar=list(fun="ns", knots=quantile(xvar, prob=c(.25,.50,.75)))
bvar <- do.call(onebasis, c(list(x=xvar), argvar))
pred.pool <- crosspred(bvar, coef=coef(mv), vcov=vcov(mv),
                       model.link="log", by=0.1)

# Center the exposure-response curve at the MMT.
mmt <- pred.pool$predvar[which.min(pred.pool$allRRfit)]
pred.pool <- crosspred(bvar, coef=coef(mv), vcov=vcov(mv),
                       model.link="log", by=0.1, cen=mmt)

# Plot pooled exposure-response curve.
plot(pred.pool, type="l", ci="n", ylab="RR",
     ylim=c(.95,1.3), lwd=2,
     xlab="Temperature (C)", main="Pooled and first-stage")
```

```
> summary(mv)
Call:  mixmeta(formula = coef ~ 1, S = vcov, method = "ml")

Multivariate random-effects meta-analysis
Dimension: 4
Estimation method: ML

Fixed-effects coefficients
    Estimate  Std. Error          z  Pr(>|z|)  95%ci.lb  95%ci.ub
b1   -0.2913      0.0196   -14.8822    0.0000   -0.3296   -0.2529  ***
b2   -0.3169      0.0207   -15.3336    0.0000   -0.3574   -0.2764  ***
b3   -0.5829      0.0464   -12.5732    0.0000   -0.6738   -0.4920  ***
b4   -0.0691      0.0314    -2.1985    0.0279   -0.1307   -0.0075    *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Random-effects (co)variance components
 Structure: General positive-definite
    Std. Dev     Corr
b1    0.0522       b1       b2       b3
b2    0.0547   0.9259
b3    0.1211   0.9280   0.7615
b4    0.0890   0.2280  -0.1427   0.5253

Multivariate Cochran Q-test for heterogeneity:
Q = 115.3949 (df = 36), p-value = 0.0000
I-square statistic = 68.8%

10 units, 4 outcomes, 40 observations, 4 fixed and 10 random-effects
parameters
  logLik       AIC       BIC
 63.5305  -99.0610  -75.4167
```

Second stage multivariate meta-analysis in R

```
# Multivariate mixed-effects meta-analysis
mv <- mixmeta(coef~1, vcov, method="ml")
summary(mv)

# Generate the temperature distribution for prediction.
bound <- rowMeans(sapply(dlist, function(x) range(x$tmean)))
xvar <- seq(bound[1], bound[2], by=0.1)

# Predicted exposure-response curve from meta-analysis estimates.
argvar=list(fun="ns", knots=quantile(xvar, prob=c(.25,.50,.75)))
bvar <- do.call(onebasis, c(list(x=xvar), argvar))
pred.pool <- crosspred(bvar, coef=coef(mv), vcov=vcov(mv),
                       model.link="log", by=0.1)

# Center the exposure-response curve at the MMT.
mmt <- pred.pool$predvar[which.min(pred.pool$allRRfit)]
pred.pool <- crosspred(bvar, coef=coef(mv), vcov=vcov(mv),
                       model.link="log", by=0.1, cen=mmt)

# Plot pooled exposure-response curve.
plot(pred.pool, type="l", ci="n", ylab="RR",
     ylim=c(.95,1.3), lwd=2,
     xlab="Temperature (C)", main="Pooled and first-stage")

# Get risk estimate for heat exposure.
xvar.heat <- quantile(xvar, prob=c(.90))
target <- as.character(round(xvar.heat))
cbind(pred.pool$allRRfit[target],
      pred.pool$allRRlow[target],
      pred.pool$allRRhigh[target])
```



Second stage multivariate meta-analysis in R

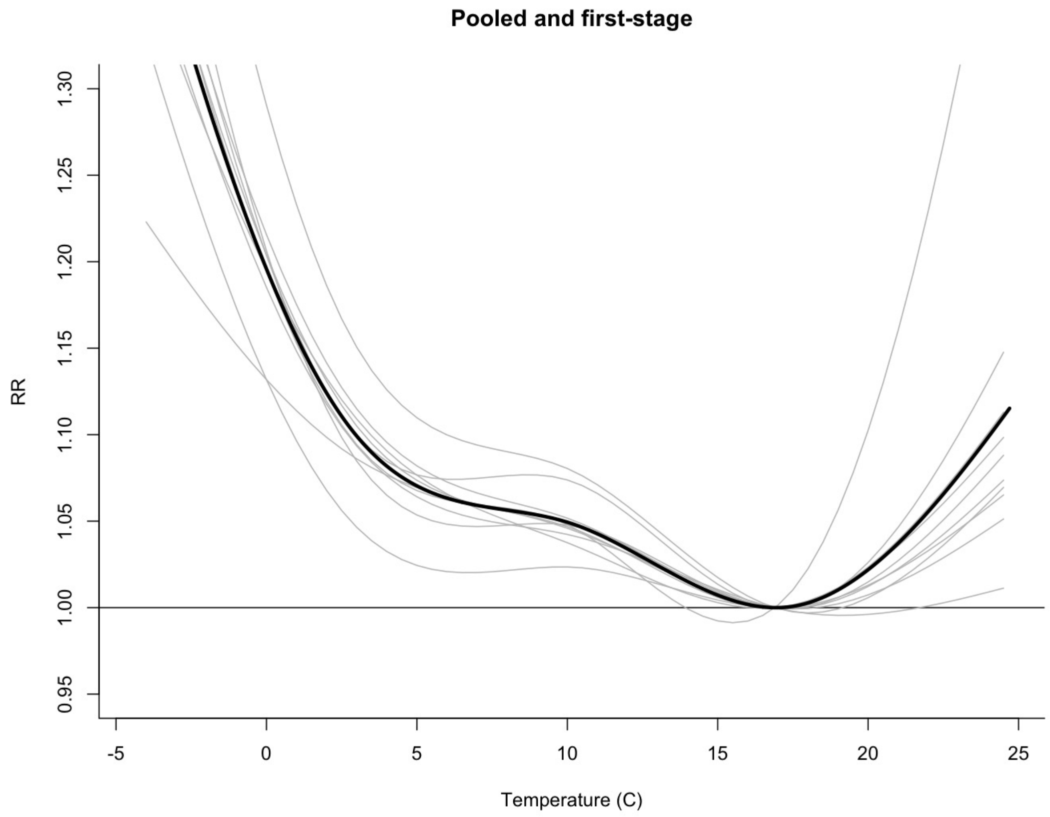
```
# Multivariate mixed-effects meta-analysis
mv <- mixmeta(coef~1, vcov, method="ml")
summary(mv)

# Generate the temperature distribution for prediction.
bound <- rowMeans(sapply(dlist, function(x) range(x$tmean)))
xvar <- seq(bound[1], bound[2], by=0.1)

# Predicted exposure-response curve from meta-analysis estimates.
argvar=list(fun="ns", knots=quantile(xvar, prob=c(.25,.50,.75)))
bvar <- do.call(onebasis, c(list(x=xvar), argvar))
pred.pool <- crosspred(bvar, coef=coef(mv), vcov=vcov(mv),
                       model.link="log", by=0.1)

# Center the exposure-response curve at the MMT.
mmt <- pred.pool$predvar[which.min(pred.pool$allRRfit)]
pred.pool <- crosspred(bvar, coef=coef(mv), vcov=vcov(mv),
                       model.link="log", by=0.1, cen=mmt)

# Plot pooled exposure-response curve.
plot(pred.pool, type="l", ci="n", ylab="RR",
     ylim=c(.95,1.3), lwd=2,
     xlab="Temperature (C)", main="Pooled and first-stage")
```

```
# Get risk estimate for heat exposure.
xvar.heat <- quantile(xvar, prob=c(.99))
target <- as.character(round(xvar.heat))
cbind(pred.pool$allRRfit[target],
      pred.pool$allRRlow[target],
      pred.pool$allRRhigh[target])
```

```
> cbind(pred.pool$allRRfit[target],
+       pred.pool$allRRlow[target],
+       pred.pool$allRRhigh[target])

       [,1]     [,2]     [,3]
24 1.098805 1.043438 1.157111
```

Second stage multivariate meta-analysis in R

```
# Predicted region-specific curve from meta-analysis estimates.
pred.reg <- lapply(seq(regions),
                   function(i) crosspred(bvar, coef=coef[i,],
                                         vcov=vcov[[i]],
                                         model.link="log",
                                         cen=mmt))

# Plot pooled exposure-response curve.
for(i in seq(regions)) lines(pred.reg[[i]], col="grey")
lines(pred.pool, lwd=3)
```



Second stage multivariate meta-regression in R

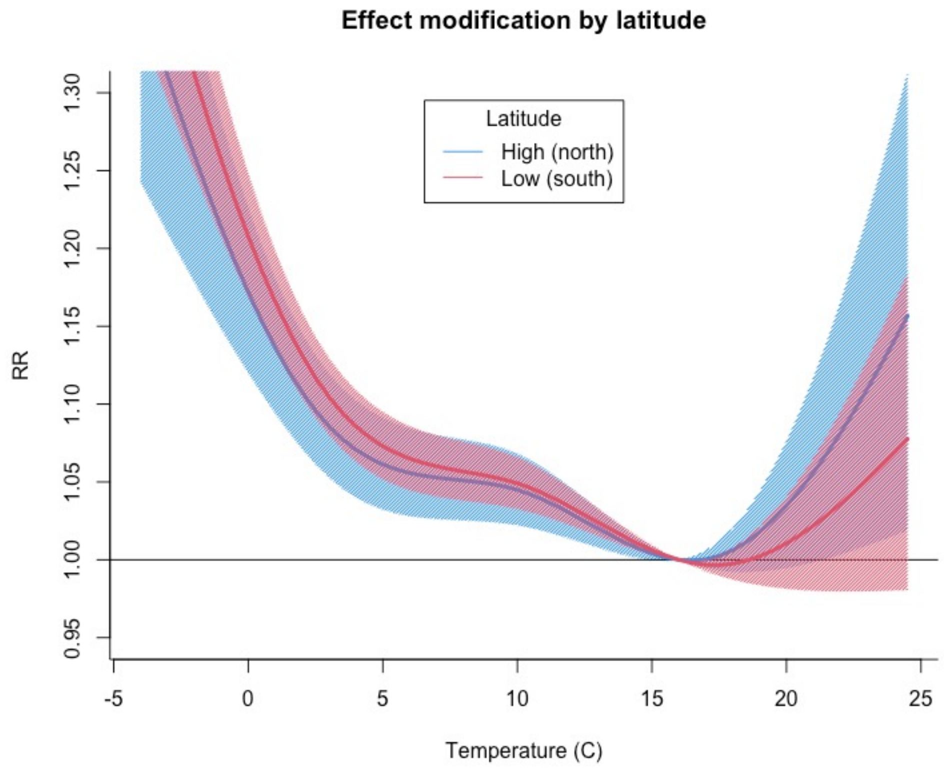
```
# Multivariate meta-regression by latitude.
mixlat <- mixmeta(coef~lat, vcov, method="ml")
print(summary(mixlat), digits=3)

# Predicted pooled exposure-response curve by latitude.
predlat <- predict(mixlat, data.frame(lat=range(lat)), vcov=T)
predmin <- crosspred(bvar, coef=predlat[[1]]$fit,
             vcov=predlat[[1]]$vcov, model.link="log", cen=16)
predmax <- crosspred(bvar, coef=predlat[[2]]$fit,
             vcov=predlat[[2]]$vcov, model.link="log", cen=16)

# Plot pooled exposure-response curve by latitude.
plot(predmax, type="l", ci.arg=list(density=50,col=4),
     ylab="RR", ylim=c(.95,1.3), col=4, lwd=3,
     xlab="Temperature (C)",
     main="Effect modification by latitude")
lines(predmin, ci="area", ci.arg=list(density=50,col=2),
      col=2, lwd=3)
legend("top", c("High (north)","Low (south)"),
       lty=1, col=c(4,2), inset=0.05, title="Latitude")
```

```
> summary(mv)
...
Fixed-effects coefficients
  b1 :
             Estimate  Std. Error       z  Pr(>|z|)  95%ci.lb  95%ci.ub
(Intercept)    -1.032       0.857  -1.205     0.228    -2.711     0.647
lat             0.014       0.016   0.865     0.387    -0.018     0.046
  b2 :
             Estimate  Std. Error       z  Pr(>|z|)  95%ci.lb  95%ci.ub
(Intercept)    -0.766       0.938  -0.816     0.414    -2.605     1.073
lat             0.009       0.018   0.479     0.632    -0.026     0.044
  b3 :
             Estimate  Std. Error       z  Pr(>|z|)  95%ci.lb  95%ci.ub
(Intercept)    -2.439       2.032  -1.200     0.230    -6.421     1.543
lat             0.035       0.039   0.914     0.361    -0.040     0.111
  b4 :
             Estimate  Std. Error       z  Pr(>|z|)  95%ci.lb  95%ci.ub
(Intercept)    -1.519       1.364  -1.114     0.265    -4.191     1.154
lat             0.028       0.026   1.064     0.288    -0.023     0.078
...

Multivariate Cochran Q-test for residual heterogeneity:
Q = 110.519 (df = 32), p-value = 0.000
I-square statistic = 71.0%

10 units, 4 outcomes, 40 observations, 8 fixed and 10 random-effects
parameters
 logLik      AIC      BIC
 64.394  -92.787  -62.387
```

Multivariate multivariate meta-regression in R

```
# Multivariate meta-regression by latitude.
mixlat <- mixmeta(coef~lat, vcov, method="ml")
print(summary(mixlat), digits=3)
```

```
# Predicted pooled exposure-response curve by latitude.
predlat <- predict(mixlat, data.frame(lat=range(lat)), vcov=T)
predmin <- crosspred(bvar, coef=predlat[[1]]$fit,
            vcov=predlat[[1]]$vcov, model.link="log", cen=16)
predmax <- crosspred(bvar, coef=predlat[[2]]$fit,
            vcov=predlat[[2]]$vcov, model.link="log", cen=16)

# Plot pooled exposure-response curve by latitude.
plot(predmax, type="l", ci.arg=list(density=50,col=4),
     ylab="RR", ylim=c(.95,1.3), col=4, lwd=3,
     xlab="Temperature (C)",
     main="Effect modification by latitude")
lines(predmin, ci="area", ci.arg=list(density=50,col=2),
      col=2, lwd=3)
legend("top", c("High (north)","Low (south)"),
       lty=1, col=c(4,2), inset=0.05, title="Latitude")
```



Limitations

- Difficult extrapolation to the local scale when combining different exposure-response functions in absolute scale (e.g., temperature in ºC)
- Comparison between cities may be tricky because of different reference values (e.g., MMT) and temperature distributions
- Modelling cities with small counts may mislead pooled estimates
- Possible solutions may be using BLUPs or analyzing with the exposure data in percentile scale

Summary

- Two–stage design with the 1^{st} and 2^{nd} stage modelling
- Combined (pooled) effect estimates across locations in multisite time-series studies
- Single and/or multiple parameters for the short-term association
- Identifying factors to explain the between-location variability

References

- Borenstein M, et al. A basic introduction to fixed-effect and random-effects models for meta-analysis. Res Sys Meth 2010:1;97-111
- Gasparrini A, Armstrong B. Multivariate meta-analysis: a method to summarize non-linear associations. Stat Med 2011:30;2504-6
- Gasparrini A, et al. Multivariate meta-analysis for non-linear and other multi-parameter associations. Stat Med 2012:31;3821-39
- Gasparrini A, Armstrong B. Reducing and meta-analysing estimates from distributed lag non-linear models. BMC Med Res Methodol 2013;13:1
- Sera F, et al. An extended mixed-effects framework for meta-analysis. Stat Med 2019:38;5429-44
- Sera F, Gasparrini A. Extended two-stage designs for environmental research. Environ Health 2022:21;41