eDNA and qPCR survey data

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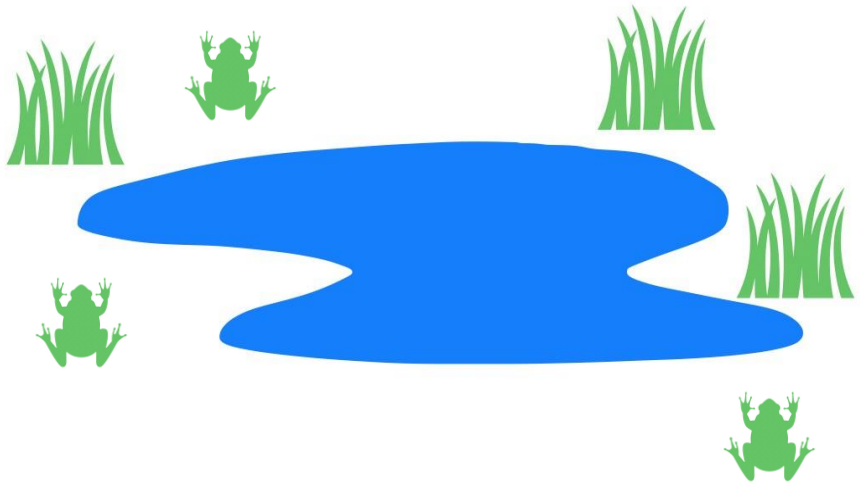
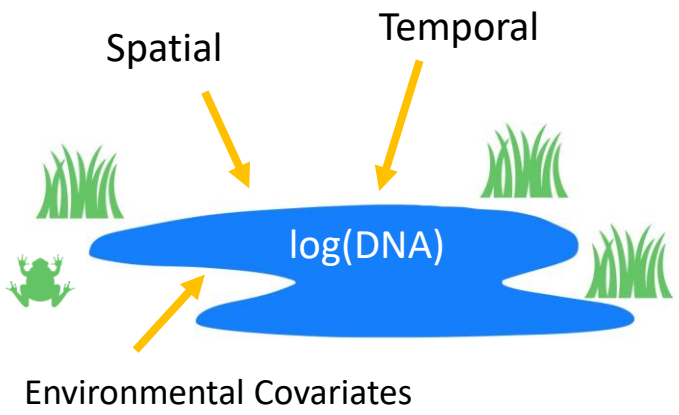
eDNA and qPCR survey data



Terminology:

eDNA: traces of DNA left by organisms in environmental samples such as water and soil

qPCR: Quantitative PCR is a technique used to amplify and quantify a target DNA molecule

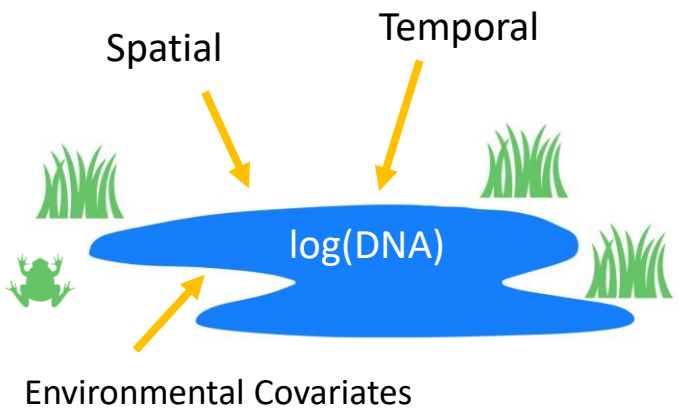


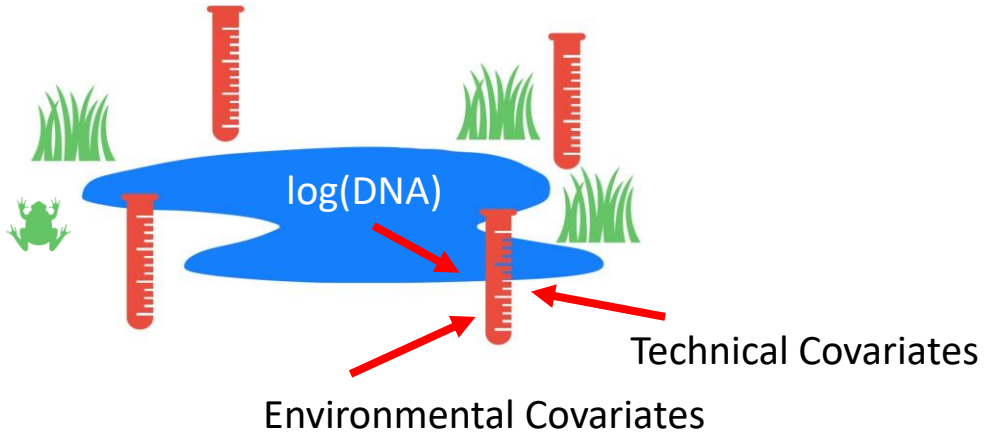
eDNA and qPCR survey data

<u>qPCR survey pipeline</u>

1. DNA availability:

   Across sites $i = 1, \dots, S$ and time-points $t = 1, \dots, T$.





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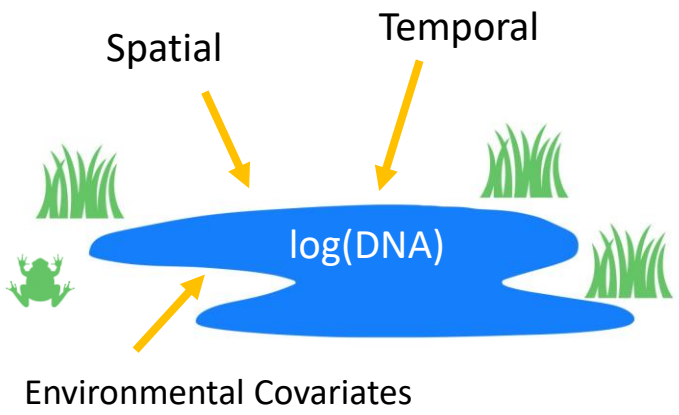

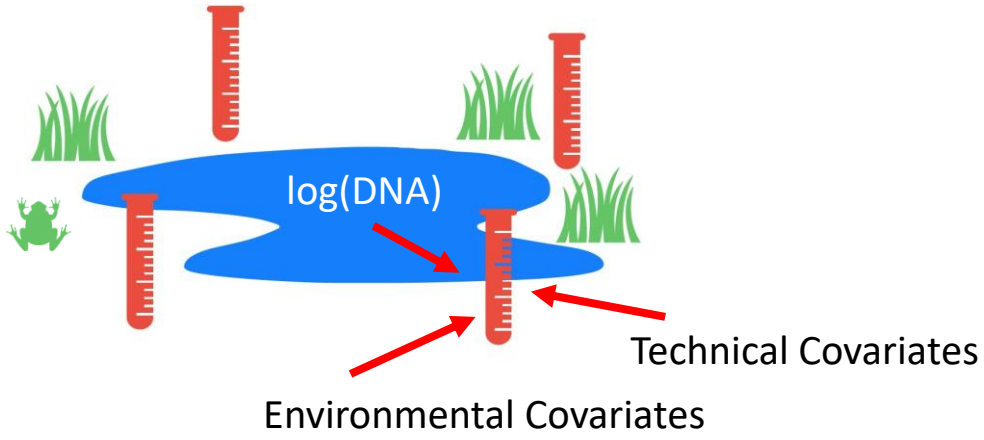

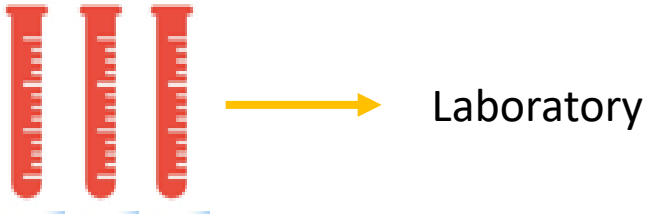

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1. DNA availability:

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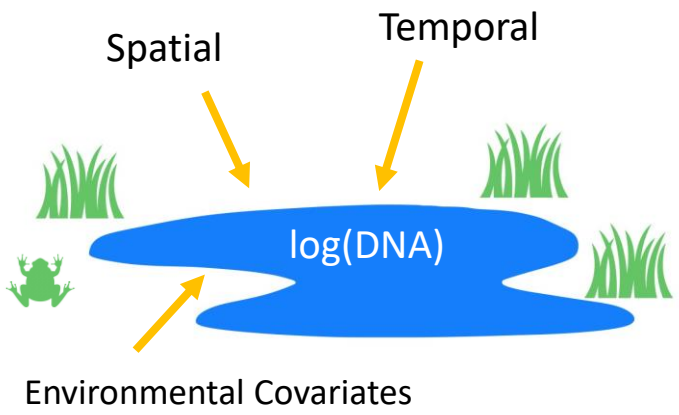
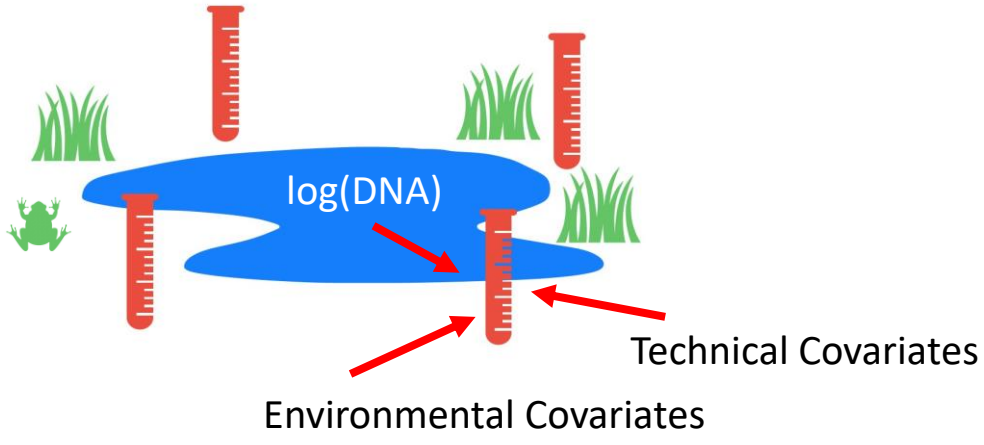
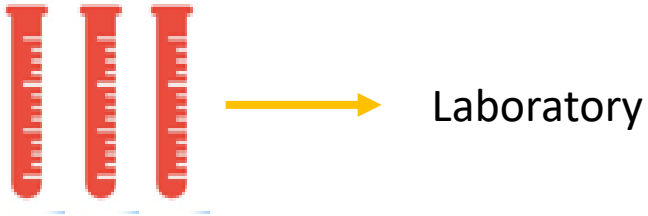
3. DNA analysis:

   Model qPCR output on DNA concentration in the sample







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   In samples $m = 1, \dots, M$.
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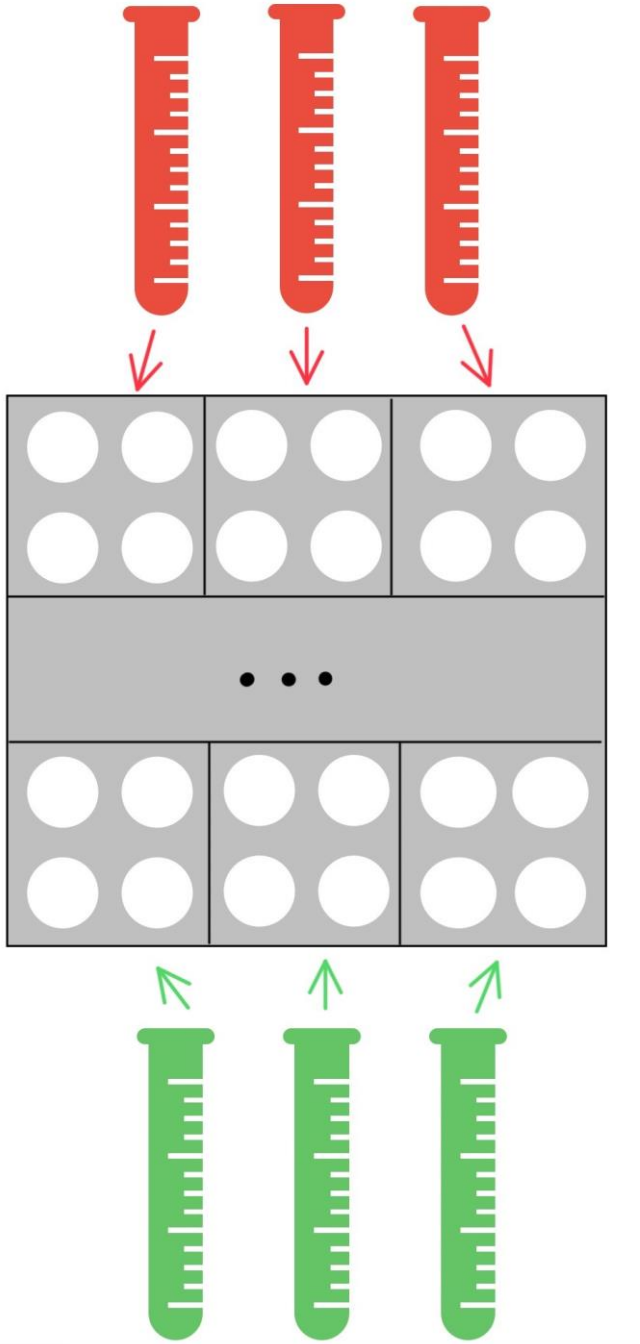
<u>Previous work:</u>

- [Espe et al. 2022] – artemis package
- [Shelton et al. 2022] – spatial model for Pacific Hake

<u>Our focus:</u>

- Modelling contamination and inhibition in the lab
- qPCR output heteroscedasticity



Collected samples

Unknown Concentrations

Plate p

K replicates

per sample

K* replicates

per standard

Standards

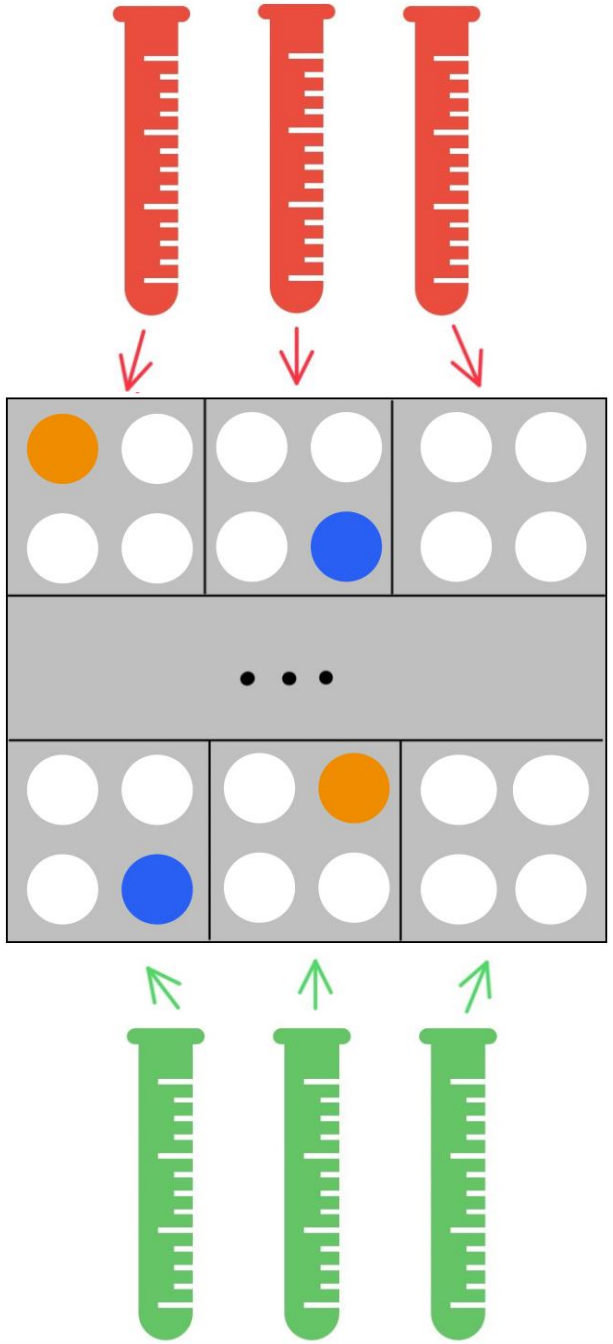
Known Concentrations

DNA analysis

Each plate $p = 1, \dots, P$ contains:

- Samples m each divided into K replicates
- **Standards** (solutions of known DNA concentration) divided into K^* replicates

DNA analysis



M samples

Unknown Concentrations

Plate p

Contaminated

Inhibited

Standards

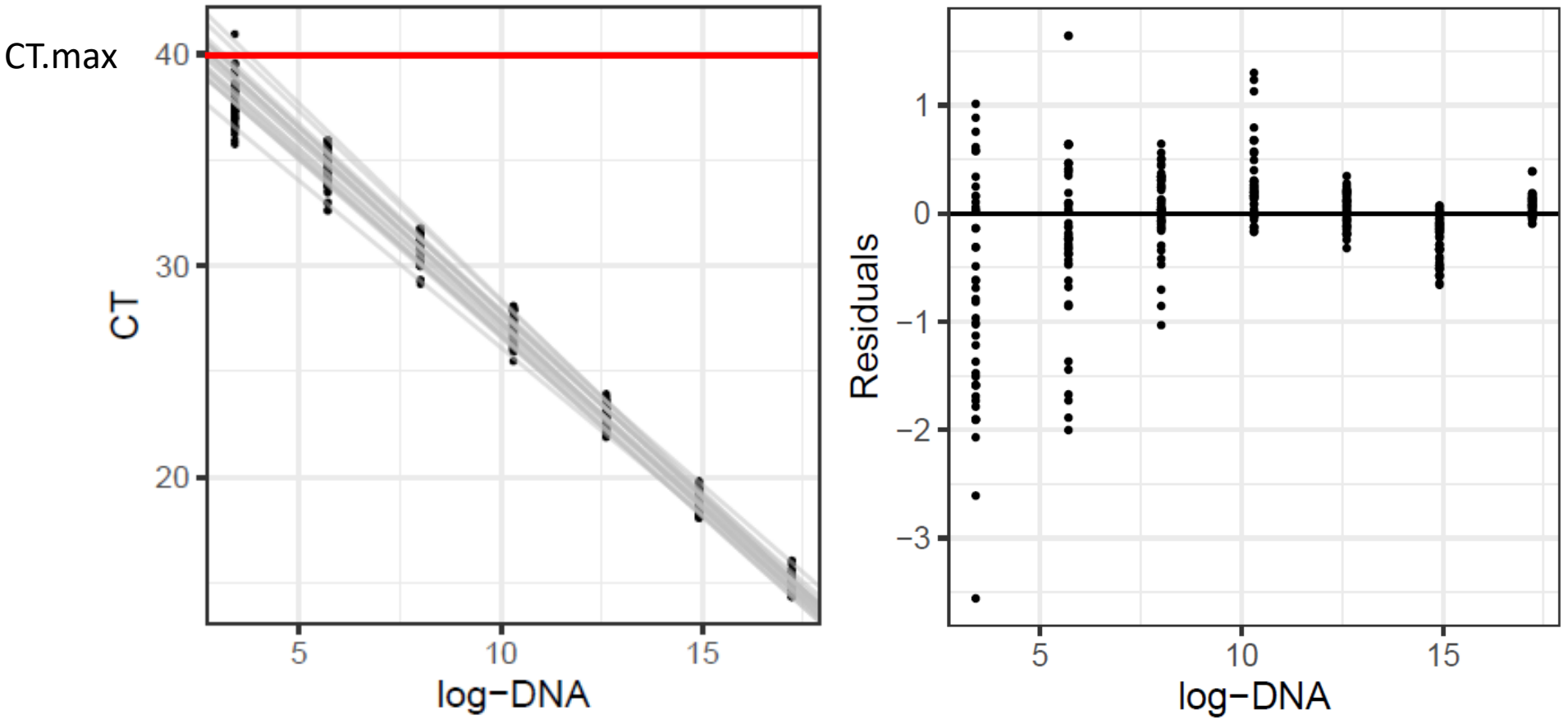
Known Concentrations

Contamination: presence of additional DNA in wells from lab through contaminated equipment or clothing

Inhibition: factors (substances or chemicals) interfering with the PCR process delaying DNA amplification

CT value – cycles to threshold, the (fractional) cycle number at which DNA is detected

DNA analysis



Heteroskedasticity: [Matz et al. 2013]

w_{imt} = DNA concentration

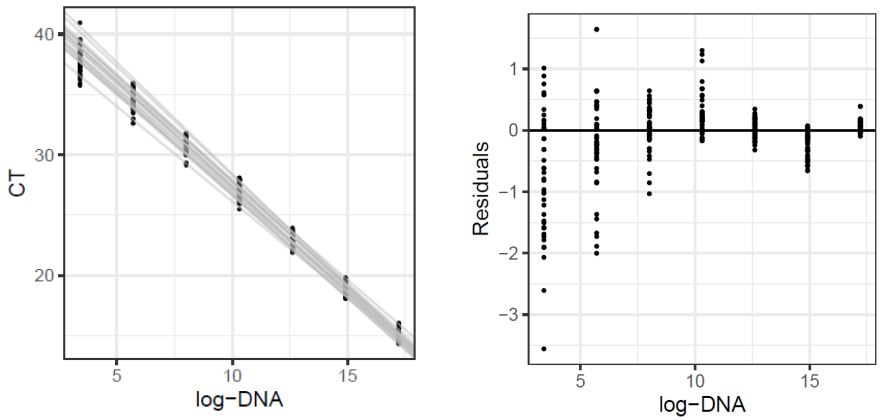
where i is the site, t is the time point,

m is the sample, and k is the replicate.

CT regression line $\mu_{imtk} = \alpha_p^1 + \alpha_p^2 \log(w_{imt}),$

CT heteroscedasticity $\sigma_y^2(w_{imt}) = \exp(a_1 + a_2 \log(w_{imt})),$

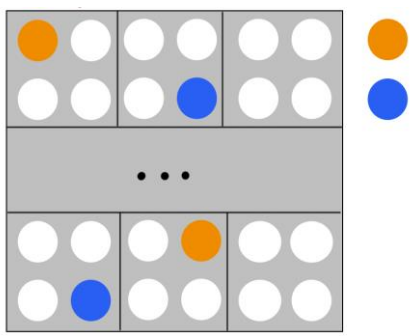
$$\alpha_p^1 \sim \mathrm{N}(\alpha_0^1, \sigma_\alpha^2)$$
$$\alpha_p^2 \sim \mathrm{N}(\alpha_0^2, \sigma_\alpha^2)$$



Status of replicate

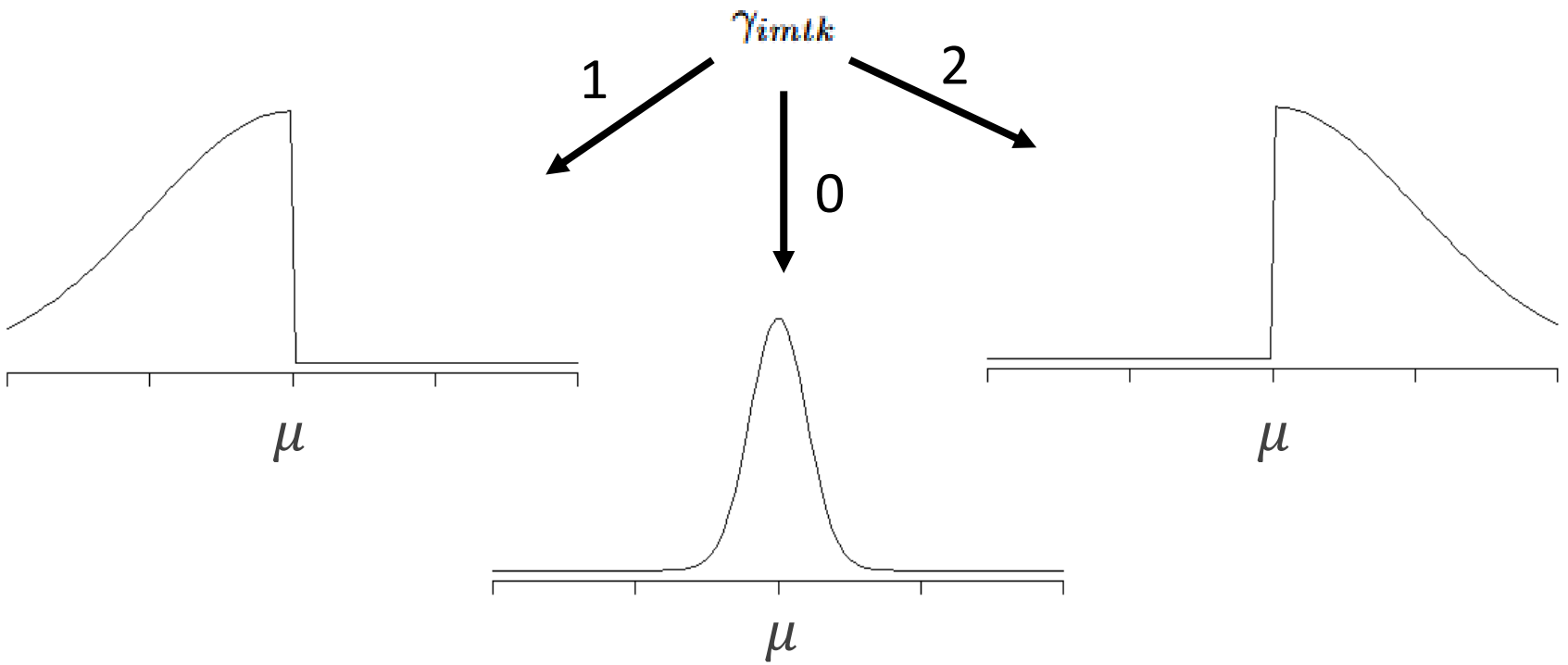
$$\gamma_{imtk} = \begin{cases} 1 & \text{contaminated with probability } p_c, \\ 2 & \text{inhibited with probability } p_h, \\ 0 & \text{neither with probability } 1 - p_c - p_h, \end{cases}$$

$$(p_c, 1 - p_c - p_h, p_h) \sim \mathrm{Dirichlet}(\pi_1, \pi_2, \pi_3)$$



Uncensored CT value

$$\tilde{C}_{imtk} \sim \begin{cases} N(\mu_{imtk}, \sigma_y^2(w_{imt})) & \text{if } \gamma_{imtk} = 0, \\ TN_{0,\mu_{imtk}}(\mu_{imtk}, \sigma_c^2) & \text{if } \gamma_{imtk} = 1, \\ TN_{\mu_{imtk},\infty}(\mu_{imtk}, \sigma_c^2) & \text{if } \gamma_{imtk} = 2, \end{cases}$$



Censored CT value

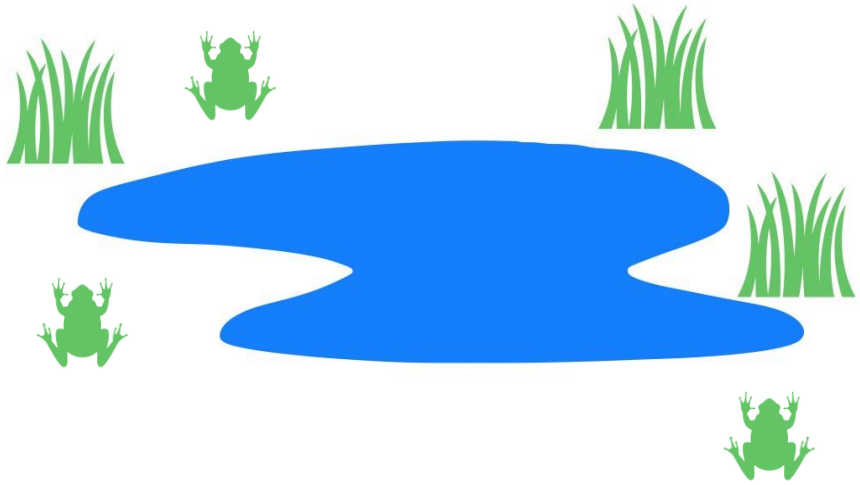
$$C_{imtk} = \begin{cases} \tilde{C}_{imtk} & \text{if } \tilde{C}_{imtk} < \text{CT.max}, \\ \text{NA} & \text{otherwise}, \end{cases}$$

Final Comments

Prior sensitivity analysis on $\alpha_{p,}^{1}$ $\alpha_{p,}^{2}$ a_1, a_2 show these are robust to prior specification.

Ignoring contamination, inhibition, and heteroscedasticity leads to increased bias and uncertainty.

Contamination/inhibition can occur at lab or DNA collection stages. This approach does not account for the collection stage.



References

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