UNIVERSITY OF COPENHAGEN
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**School Of Geosciences**

# COMPARISON OF TOP-DOWN AND BOTTOM-UP APPROACHES ON SPECIFIC LEAF AREA PATTERNS, AT GLOBAL, LATITUDINAL, AND BIOME SCALES

By

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in partial fulfilment of the requirement
for the degree of BSc with Honours
in Ecological and Environmental Sciences

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## Abstract

(the spacing is set to 1.5)

no more than 250 words for the abstract

- a description of the research question/knowledge gap – what we know and what we don't know
- how your research has attempted to fill this gap
- a brief description of the methods
- brief results
- key conclusions that put the research into a larger context

# Contents

# Acknowledgements

Thank you for following this tutorial!

I hope you'll find it useful to write a very professional dissertation.

# 1 Introduction

- introduce the reader to the subject area and clarify the knowledge gap that the dissertation research will fill.
- set the context for the dissertation by reviewing the relevant literature.
- include relevant references to general (theoretical papers and reviews) and specific (specific to the particular question addressed) literature, to justify the research that has been undertaken and define the questions being addressed.
- state the primary research questions and hypotheses in the final paragraph.
- follow an 'inverted triangle' format, progressing from general scientific ideas and why they matter to the specific research questions addressed in the dissertation project.

*The introduction should not be just a 'Literature Review'.*

(Breton et al. n.d.)

(Breton, Diamond & Kress n.d.)

Breton et al. (n.d.)

Breton, Diamond & Kress (n.d.)

(Martin n.d., Breton et al. n.d.)

Breton et al.

Breton, Diamond & Kress

n.d.

(n.d.)

# 2 Methods

Write your methods here. In this tutorial you can use this already made file\to add examples of figures and tables and explore knitr and kableExtra functionalities!

# 3 Results

```
#____Separate into groups ______
AB_FF <- nrow(preexp_1 %>%
  filter(group2 == "AB-FF"))

AB_BF <- nrow(preexp_1 %>%
  filter(group2 == "AB-BF"))

CON_FF <- nrow(preexp_1 %>%
  filter(group2 == "CON-FF"))

CON_BF <- nrow(preexp_1 %>%
  filter(group2 == "CON-BF"))
#____ ____#
```

The main goal of this thesis project was to create a NEC mouse model by combining Maternal Antibiotics Treatment with Formula-Feeding. This hypothesis was tested in a series of experiments (Exp 1-4), each successive experiment building on the findings from the former. The following is a description of each experiment. An abridged version of each experiment is provided, followed by a detailed description of experimental setup and results.

| Proinflammatory cytokines | | | | | |
|---|---|---|---|---|---|
| | Treatment groups (N total = 30) | | | | |
| Cytokines | **AB-BF**, N = 8[1] | **AB-FF**, N = 11[1] | **CON-BF**, N = 8[1] | **CON-FF**, N = 3[1] | **p-value**[2] |
| IL-10 | 1.32 (1.2, 1.6) | 0.00 (0.0, 0.5) | 1.16 (0.7, 1.8) | 2.12 (1.2, 2.3) | 0.012 |
| IL-12p70 | 13.60 (10.4, 18.7) | 15.96 (9.4, 17.0) | 10.30 (7.4, 12.4) | 14.32 (14.1, 15.8) | 0.3 |
| IL-1b | 3.81 (2.8, 4.6) | 2.64 (2.4, 3.9) | 2.61 (2.0, 6.6) | 3.10 (2.5, 9.1) | >0.9 |
| IL-2 | 1.41 (1.3, 1.7) | 1.22 (1.1, 1.3) | 1.46 (1.3, 1.6) | 1.14 (1.0, 1.3) | 0.053 |
| IL-4 | 0.98 (0.9, 1.1) | 0.52 (0.5, 0.6) | 0.72 (0.6, 1.0) | 0.77 (0.7, 0.9) | 0.004 |
| IL-5 | 2.01 (1.5, 2.2) | 0.89 (0.6, 1.1) | 1.72 (1.6, 2.1) | 1.34 (1.2, 1.7) | <0.001 |
| IL-6 | 14.97 (14.1, 17.9) | 24.06 (21.8, 30.2) | 12.34 (10.8, 16.7) | 39.42 (32.2, 40.6) | 0.002 |
| KCGRO | 7.76 (5.7, 10.4) | 7.20 (6.0, 9.4) | 8.09 (4.5, 25.1) | 8.91 (7.9, 17.6) | 0.9 |
| TNFa | 2.64 (2.4, 3.3) | 1.45 (1.4, 1.8) | 2.98 (2.2, 3.7) | 1.84 (1.5, 2.4) | 0.004 |

[1] Median (IQR)
[2] Kruskal-Wallis rank sum test
* P-value indicates overall group differences
**Matrinem round 1**

Figure 1: Experimental protocols

## 3.1 Exp 1: Model feasibility

### 3.1.1 Rationale:

- *Chen et al.*, showed that treatment with antibiotics during pregnancy(Maternal Antibiotics Treatment, **MAT**) "resulted" in mild NEC-like symptoms in the offspring.
- XXX *[Find paper]* found formula-feeding to be damaging to the intestine.
- One of the primary causal factors of NEC is formula-feeding (paper on what is thought to cause NEC).
- We tested the hypothesis that MAT combined with formula feeding (**FF**) would result in a worsened phenotype - even more NEC-like than either treatment on it's own.

### 3.1.2 Aim:

- To test whether it was possible to keep mice alive from postnatal day 3 and 48 hours onwards, while being separated from the mother.
- Whether combining a broad spectrum antibiotic with formula-feeding will result in NEC-like symptoms such as:

- Visible signs of intestinal inflammation and / or greater levels of pro-inflammatory cytokines expressed in tissue or serum

#### 3.1.3 Conclusion:

- Survival rate: 29 of 38(76.3157895%)
- Visual inspection: no clear signs of intestinal inflammation
- Cytokine expression: Statistically significant differences in the level of STATISTICAL-SIGNIF-CYTOKINE-NAMES.

#### 3.1.4 Detailed description of Exp 1. Date of exp

*Antimals and experimental setup* \ - The experimental setup has been described in detail in the "Animal experimental setup" methods section. Detials specific for Exp-1 are outlined below. \ - Six male and 18 female C57BL/6N mice were co-housed for 24 hours in 6 cages (1 male + 3 female each). - Two weeks after mating, pregnant females were separated evenly into maternal antibiotic treatment (MAT) and water-only controls (CON). - Antibiotics (Gentamicin, Vancomycin, Neomycin, and Ampicillin (all 0.5 g/L)) were mixed into the drinking water of the MAT mice while controls received water only. - Antibiotic treatment commenced on gestational day 15 and continued until delivery. - Each mother was housed with her offspring in separate cages during the first 2 days after birth. - On day 3, mice from water or antibiotics treated mothers were separated and assigned to either formula feeding (FF) or breastfeeding (BF) forming four experimental groups: AB + formula-feeding (AB-FF, N = 13 ) AB + breastfeeding (AB-BF, N = 8 ), Water + formula-feeding (CON-FF, N = 9 ), and CON + breastfeeding (CON-BF, N = 8 ). - Formula-feeding was performed as per METHODS AND MATERIALs. In Exp-1 we administered 0.08 ml formula for pups over 2g, and 0.06 for pups less 2g every 3 hours throughout the entire study. In later experiments this dose was changed, and feeding intervals were increased to every 4 hours between midnight and 08 AM. \ - 29 of 38 pups survived the treatments. - Those that did not survive were euthanize because of esophageal perforation during feeding. - After fine-tuning of the feeding methods we were able to feed the pups without further harm. - We never had to euthanize a pup that reached our humane endpoints, since all the surviving pups were in generally good shape. - While significantly lighter than their

breastfed controls, all pups increased their bodyweight during the experiment, attesting to the efficacy of our feeding regiment. - The pups were physically active and responded to changes in light and physical stimuli. - Skin color remained bright purple and fur development was visible throughout the experiment. - No signs of intestinal inflammation were evident from the visual inspection of intestines during sampling. Our subjective observation of the intestines were small degrees of bloating in formula-fed pups.

# 4 Discussion

the purpose of the discussion is to summarise your major findings and place them in the context of the current state of knowledge in the literature. When you discuss your own work and that of others, back up your statements with evidence and citations. - The first part of the discussion should contain a summary of your major findings (usually 2 – 4 points) and a brief summary of the implications of your findings. Ideally, it should make reference to whether you found support for your hypotheses or answered your questions that were placed at the end of the introduction. - The following paragraphs will then usually describe each of these findings in greater detail, making reference to previous studies. - Often the discussion will include one or a few paragraphs describing the limitations of your study and the potential for future research. - Subheadings within the discussion can be useful for orienting the reader to the major themes that are addressed.

# 5 Conclusion

The conclusion section should specify the key findings of your study, explain their wider significance in the context of the research field and explain how you have filled the knowledge gap that you have identified in the introduction. This is your chance to present to your reader the major take-home messages of your dissertation research. It should be similar in content to the last sentence of your summary abstract. It should not be a repetition of the first paragraph of the discussion. They can be distinguished in their connection to broader issues. The first paragraph of the discussion will tend to focus on the direct scientific implications of your work (i.e. basic science, fundamental knowledge) while the conclusion will tend to focus more on the implications of the results for society, conservation, etc.

Waiting for the command to add the references...

# 6 Bibliography

Breton, A. R., Diamond, A. W. & Kress, S. W. (n.d.), 'Encounter, survival, and movement probabilities from an atlantic puffin (fratercula arctica) metapopulation', **76**(1), 133–149. _eprint: https://esajournals.onlinelibrary.wiley.com/doi/pdf/10.1890/05-0704.
**URL:** *https://esajournals.onlinelibrary.wiley.com/doi/abs/10.1890/05-0704*

Martin, A. R. (n.d.), 'The diet of atlantic puffin *Fratercula arctica* and northern gannet *Sula bassana* chicks at a shetland colony during a period of changing prey availability', **36**(3), 170–180.
**URL:** *http://www.tandfonline.com/doi/full/10.1080/00063658909477022*

# 7 Appendix(ces)

## 7.1 Appendix A: additional tables

## 7.2 Appendix B: additional figures

## 7.3 Appendix C: code

```
knitr::include_graphics("img/sund.pdf")
library(knitr)  # for dynamic report generation
library(kableExtra) # to build complex HTML or 'LaTex' tables
library(readxl)
library(tidyverse)
library(broom)
library(ggpubr)
library(patchwork)
#____Load overview of sample IDs______
pups <- read_excel("../data/processed/weight_development.xlsx")

#____ Separate into experimental rounds ____#
preexp_1 <- pups %>%
  filter(exp_number == "1")

exp1_survived <- preexp_1 %>%
  filter(!is.na(bodyweight_baseline_plus_48h)) %>%
  nrow()
#____ ____#
#____Separate into groups ______
AB_FF <- nrow(preexp_1 %>%
  filter(group2 == "AB-FF"))

AB_BF <- nrow(preexp_1 %>%
  filter(group2 == "AB-BF"))

CON_FF <- nrow(preexp_1 %>%
  filter(group2 == "CON-FF"))
```

```r
CON_BF <- nrow(preexp_1 %>%
  filter(group2 == "CON-BF"))
#____ ____#



include_graphics("../results/round_1/Table cytokines round 1.png")
#____Load cytokine data______
cytokines <- read_excel("../data/processed/weight_development.xlsx")

#____ Separate into experimental rounds ____#
preexp_1 <- pups %>%
  filter(exp_number == "1")

exp1_survived <- preexp_1 %>%
  filter(!is.na(bodyweight_baseline_plus_48h)) %>%
  nrow()
#____ ____#
# this code chunk displays all source code from your entire dissertation document (tha
```