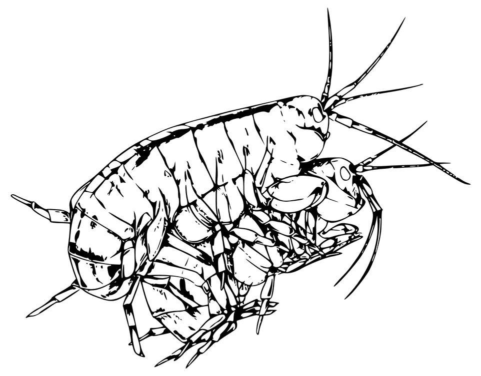
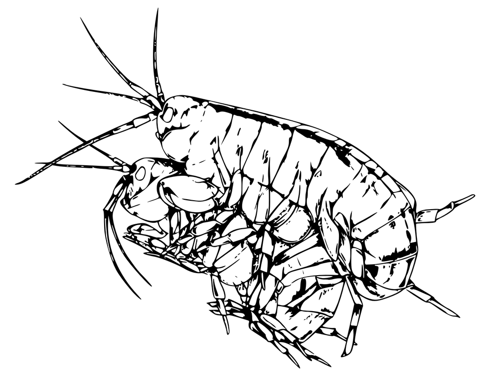


A study of germ layer specification of the crustacean *Parhyale hawaiensis*

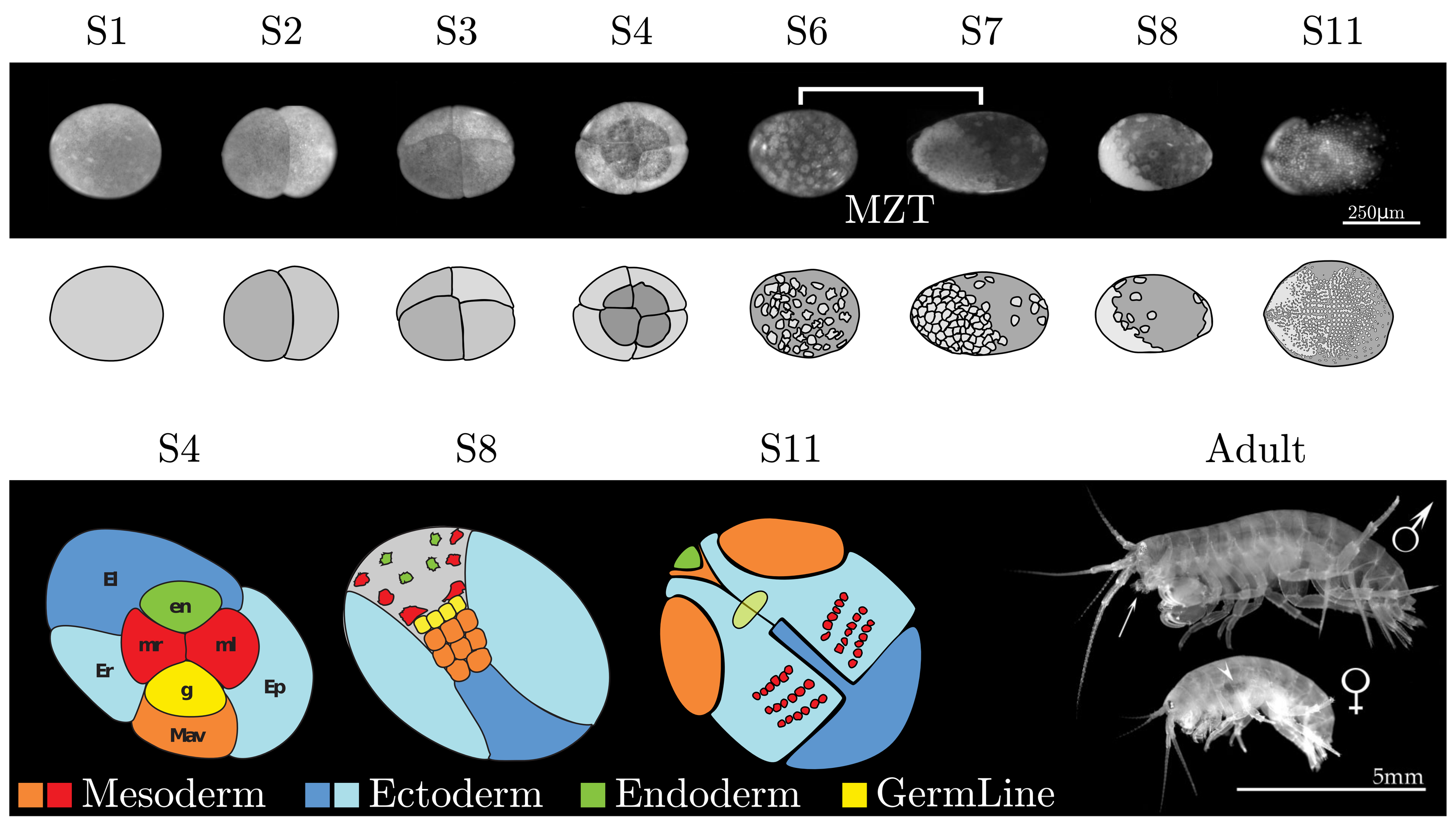


Leo Blondel, Cassandra G. Extavour

Introduction

Here I study the germ layer specification of an emerging model organism, the crustacean *Parhyale hawaiensis*, by using single cell RNA sequencing to quantify the gene expression landscape of embryonic development from the single cell stage to the establishment of major body plan features.
Parhyale hawaiensis possesses a well defined genome [1], germ layers entirely derived from a maximum of three cell progenitors [2], a small number of cells at gastrulation, a well defined fate map of all germ layers, ease of culture and embryo collection, and ease of cell harvesting. This approach will allow me to explore the topologies, shifts and rearrangements of gene regulatory networks along the epigenetic landscape. Further, I will analyze the changes in mRNA expression landscape post MZT, notably the order and timing of gene expression, and their association with the different germ layers.

Parhyale hawaiensis



It's a crustacean amphipod first described on the coast of Hawaii. It's embryogenesis happens over the course of 10 days. Here are selected stages of embryogenesis showing the first holoblastic cleaveages (S1-S4), gastrulation (S8) and the formation of the germ band (S11). [3]
Along with the micrographs are represented the known fate map. Cells are commited to a germ layer at the 8 cell stage.
Multiple key features make this animal the perfect model organism to study cell fate determination along with the MZT:

- Holoblastic cleavages allowing for single cell isolation
- Cell fate commitment at the 8 cells stage
- Low number of cells at the germ band stage
- Well annotated genome
- Ease of culture

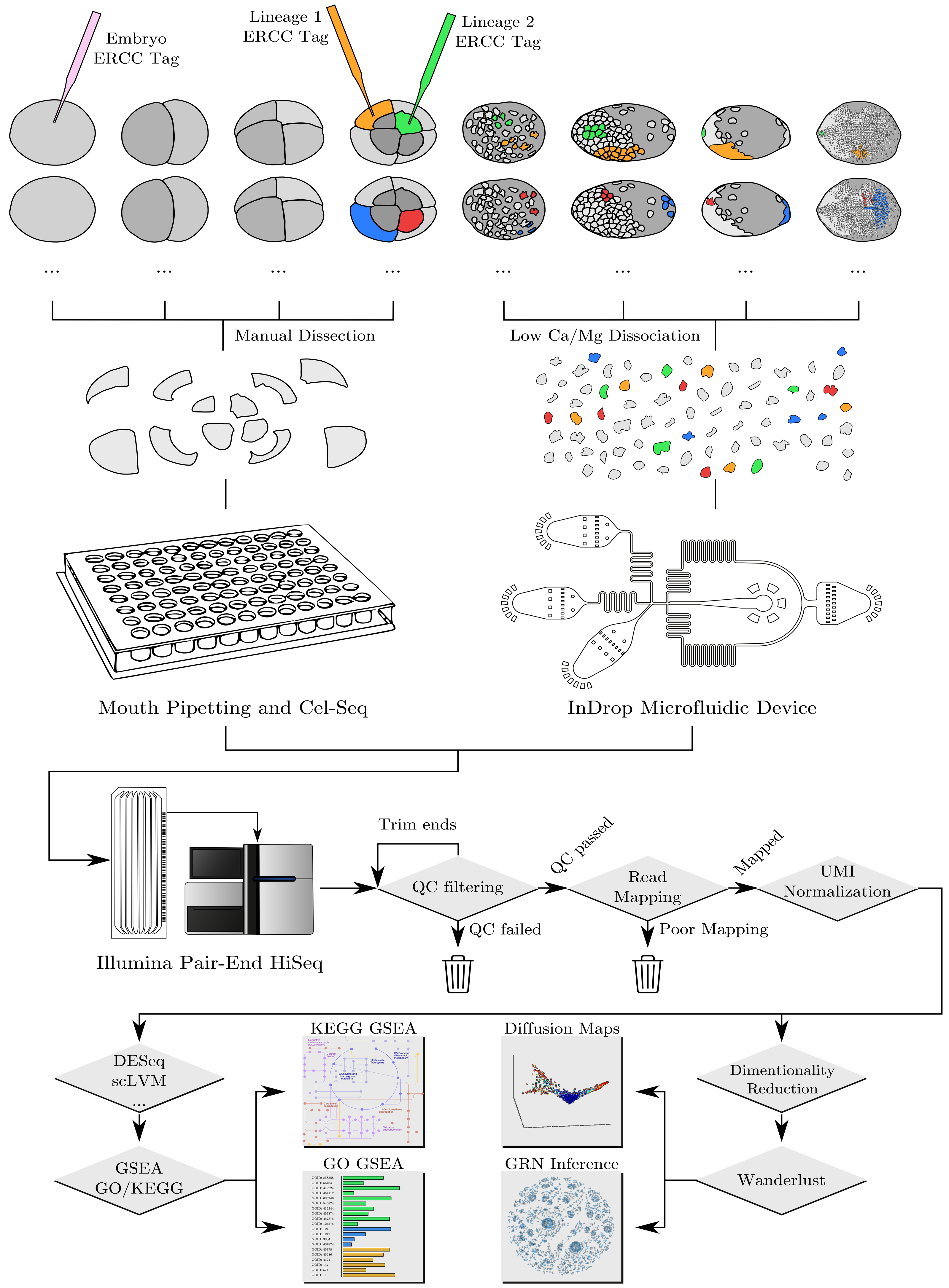
Questions

What is the exact timing of the MZT ?

What is the relationship between the mozaic RNAPolII expression and cell lineage ?

Is there different activated programs for each Germ Layer ?

Experimental Layout



Maternal to Zygotic Transition

Previous research has shown mosaic expression of RNA Polymerase II as early as the 32 cell stage and ubiquitous expression at the 128 cell stage [4]. This hints that the MZT in *Parhyale hawaiensis* happens in between the 32 cell stage and 128 cell stage.
Using single cell RNA sequencing data, I will be able to analyze the exact timing of the MZT at a single cell resolution.
Moreover, there seems to be a homolog of the drosophila MZT master regulator Zelda in *Paryhale hawaiensis* genome (gene homology search in published genome [Leo Blondel, unpublished observation]).

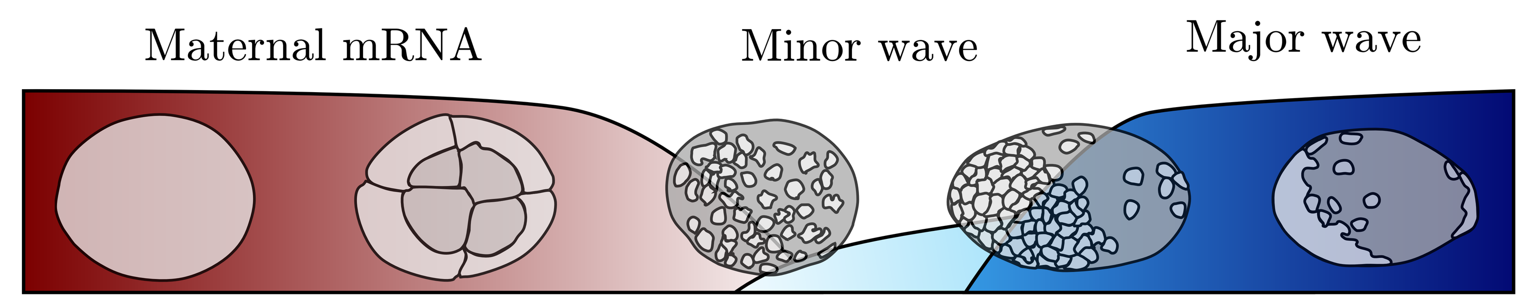
Hypotheses

1) *Parhyale hawaiensis* MZT will be similar to other animals and be composed of two waves of transcription, minor and major[5].

2) The gene Zelda plays a key role in the regulation of the minor wave of transcription.

3) The minor wave of new transcripts will be detected around the 32 cell stage.

4) The major wave of new transcripts will occur 1 or 2 divisions later, between the 64 and 128 cell stage.



Stage	S1	S4	S6	S7	S8
Cleavage	0	3	5	7	9
Time (h)	0	8	12	18	24

Credits

[1] Gerberding, M., Browne, W. E., & Patel, N. H. (2002). Cell lineage analysis of the amphipod crustacean Parhyale hawaiensis reveals an early restriction of cell fates. Development (Cambridge, England), 129(24), 5789–5801. https://doi.org/10.1242/dev.00155
[2] Kao, D., Lai, A. G., Stamataki, E., Rosic, S., Konstantinides, N., Jarvis, E., ... Aboobaker, A. (2016). The genome of the crustacean parhyale hawaiensis, a model for animal development, regeneration, immunity and lignocellulose digestion. eLife, 5 (November 2016), 1–45. https://doi.org/10.7554/eLife.20062.001
[3] Browne, W. E., Price, A. L., Gerberding, M., & Patel, N. H. (2005). Stages of embryonic development in the amphipod crustacean,Parhyale hawaiensis. Genesis, 42(3), 124–149. https://doi.org/10.1002/gene.20145
[4] Nestorov, P., Battke, F., Levesque, M. P., & Gerberding, M. (2013). The Maternal Transcriptome of the Crustacean Parhyale hawaiensis Is Inherited Asymmetrically to Invariant Cell Lineages of the Ectoderm and Mesoderm. PLoS ONE, 8(2), e56049. https://doi.org/10.1371/journal.pone.0056049
[5] Tadros, W., & Lipshitz, H. D. (2009). The maternal-to-zygotic transition : a play in two acts. Development, 136, 3033–3042. https://doi.org/10.1242/dev.033183

Research performed in the Extavour laboratory, Organismic and Evolutionary Biology (OEB) and Mollecular and Cellular Biology (MCB), Harvard University

lblondel@g.harvard.edu

mco
molecules, cells and organisms

HARVARD
UNIVERSITY