Leveraging Zebrafish to Identify Chemicals Disrupting Early Embryonic Development

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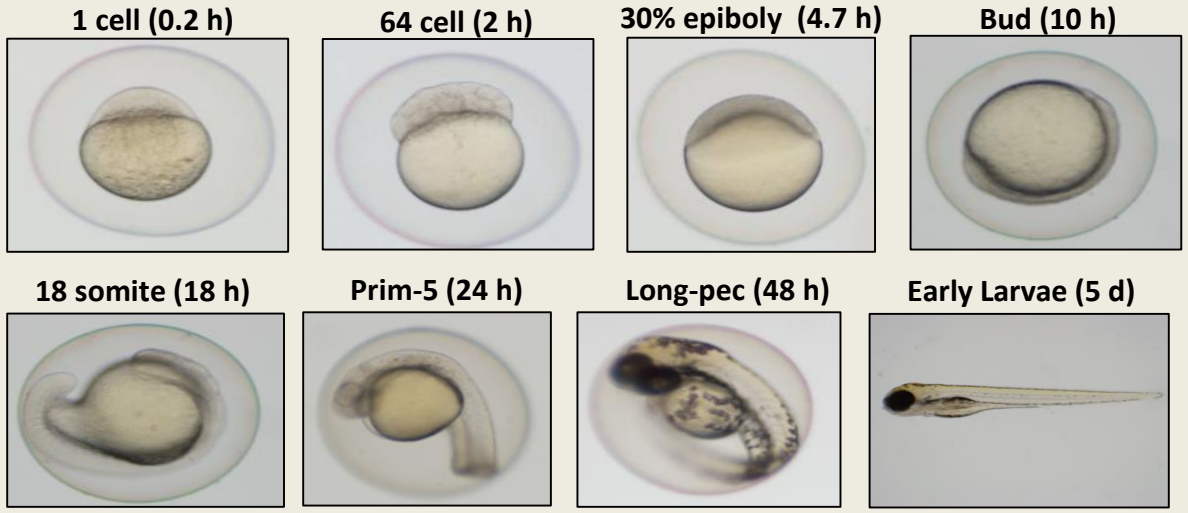
The Zebrafish Embryo and Toxicity Testing

- Due to the limitations of toxicity testing within mammalian species, alternatives models and assays are needed to support the rapid prioritization of chemicals, mixtures, and environmental samples
- This is particularly true for industrial chemicals, for which there is minimal developmental toxicity data available.
- Zebrafish embryos offer a promising cost-effective vertebrate model to support toxicity testing.



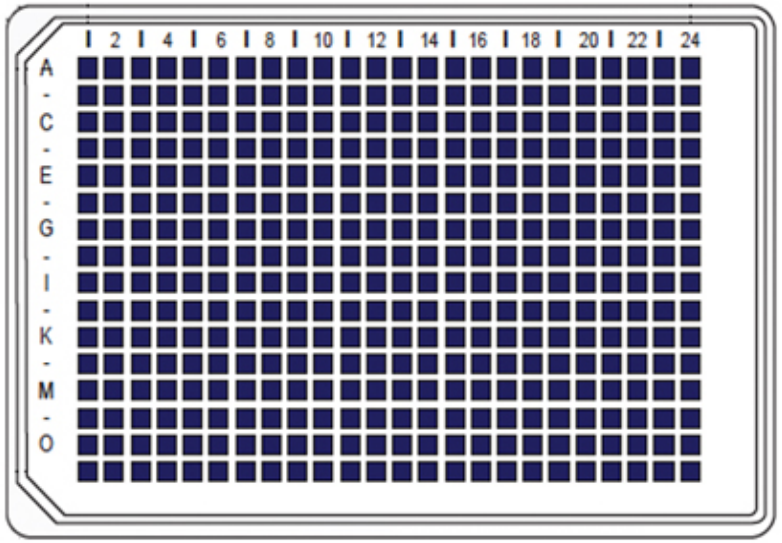
- Transparent, *Ex utero* development
- Rapid developmental timeline
- High Fecundity
- Small Size

High-Content Screening Assay

1 Embryos per well, 16 Embryos per treatment, 40 Compounds + 4 control columns per day

Static exposure (5-25 hpf or 23-25 hpf)

384-well glass-bottom plate
One embryo per well; 50 µl per well
N=16 embryos per treatment group



Treatment groups per plate:
Vehicle control (0.1% DMSO) (2 columns)
Positive control (6.25 µM Abamectin) (2 columns)
20 LOPAC^{1280} compounds (10 µM) (20 columns)

Images

2X objective
Four wells imaged at once
18-s time-lapsed acquistion
60 frames per acquisition

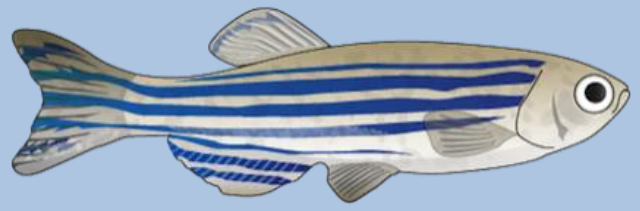
Imaging duration: ~30 min
Images per plate: 23,040
AVIs per plate: 384

Endpoints

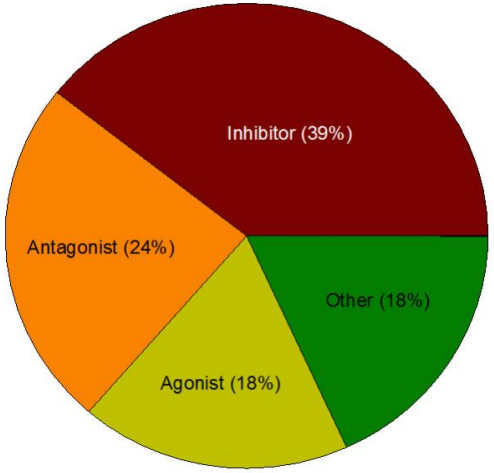
Project Objectives

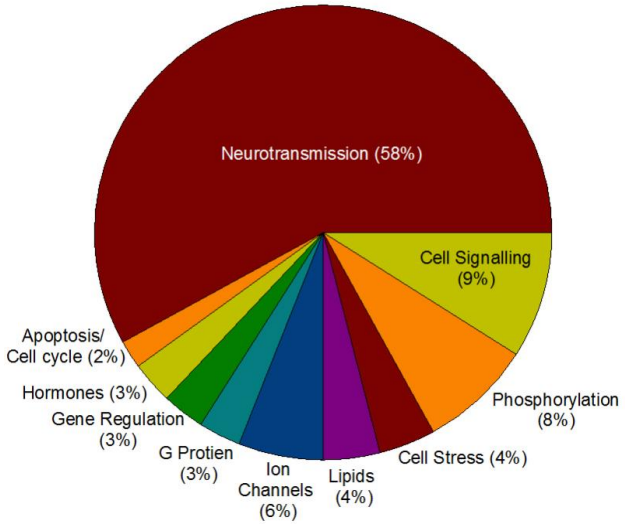
1. Using our validated zebrafish screening assay, screen a large, well-characterized library of compounds and identify the assay's predictability.

1. Conduct hypothesis-driven mechanistic investigations of a compound hit.



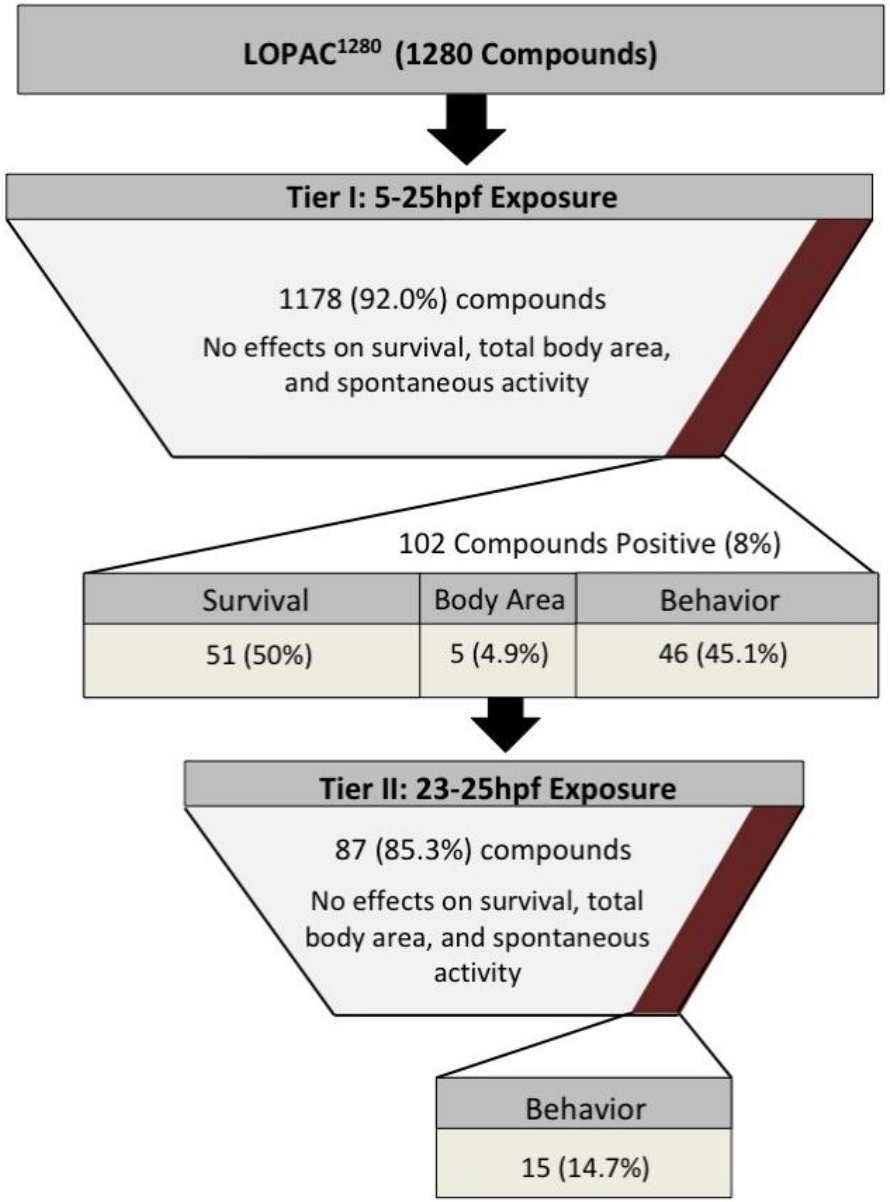
Objective 1: LOPAC^{1280} Compound Screen





Library of Pharmacologically Active Compounds (LOPAC^{1280})

- Commercially available
- Widely used for high-throughput assay validation.
- Well-characterized small molecules
- Diverse set of biological receptors and modes of action



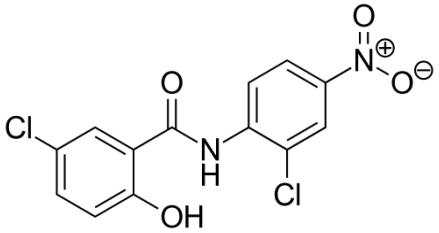
S. Vliet et al., 2017

Objective 2: Niclosamide

Tier I 20 hour Exposure 5-25 hpf	10 µM →	100 % Embryo Mortality
Tier II 2 hour Exposure 23-25 hpf	10 µM →	100 % Behavioral Alterations (Hypoactivity)

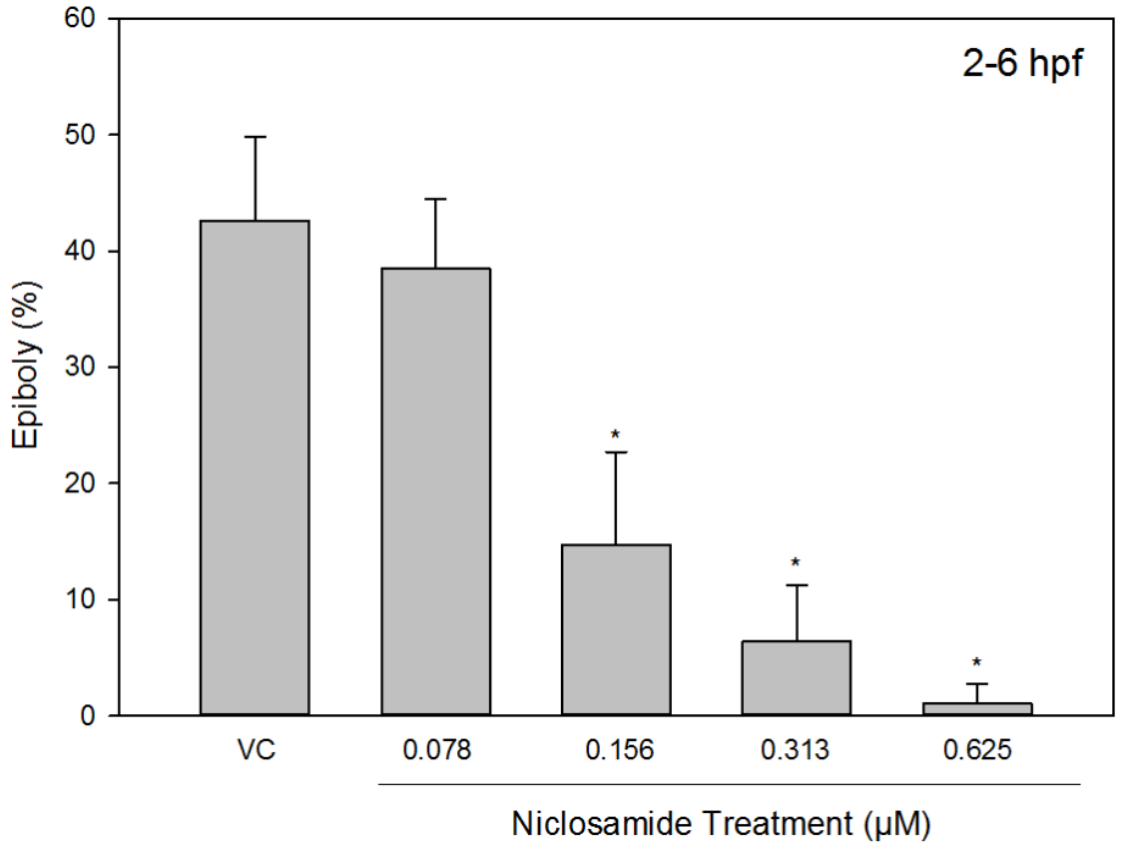
- Widely used used for treatment of tapeworm in human, veterinary, and livestock applications (WHO List of Essential Medicines).

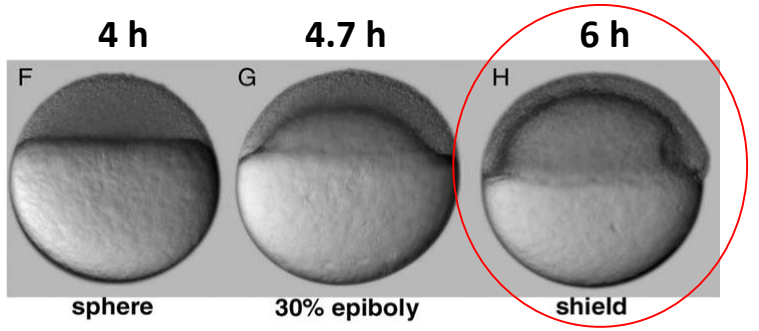
- Under investigation for a variety of alternative human-health applications.
- Developmental Toxicity for alternative uses is not well evaluated.

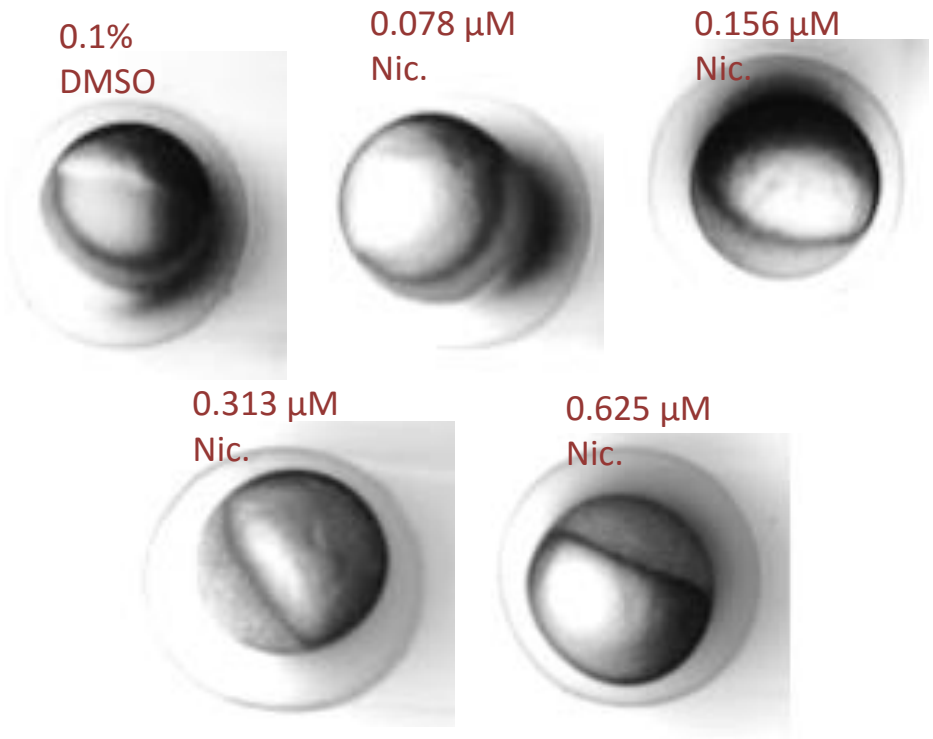


Product Images: indiamart.com

Niclosamide: Developmental Toxicity







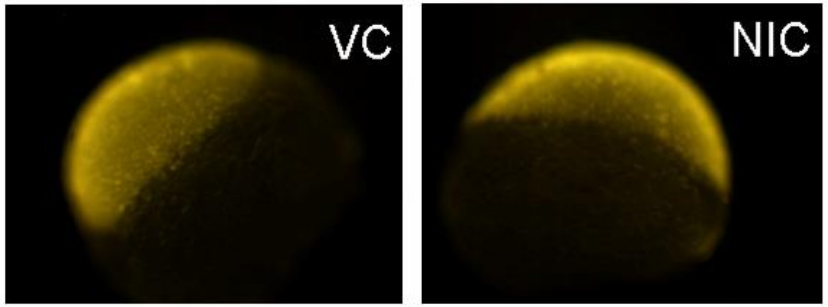
Exposure to niclosamide induces a concentration-dependent delay in early embryonic development (epiboly progression).

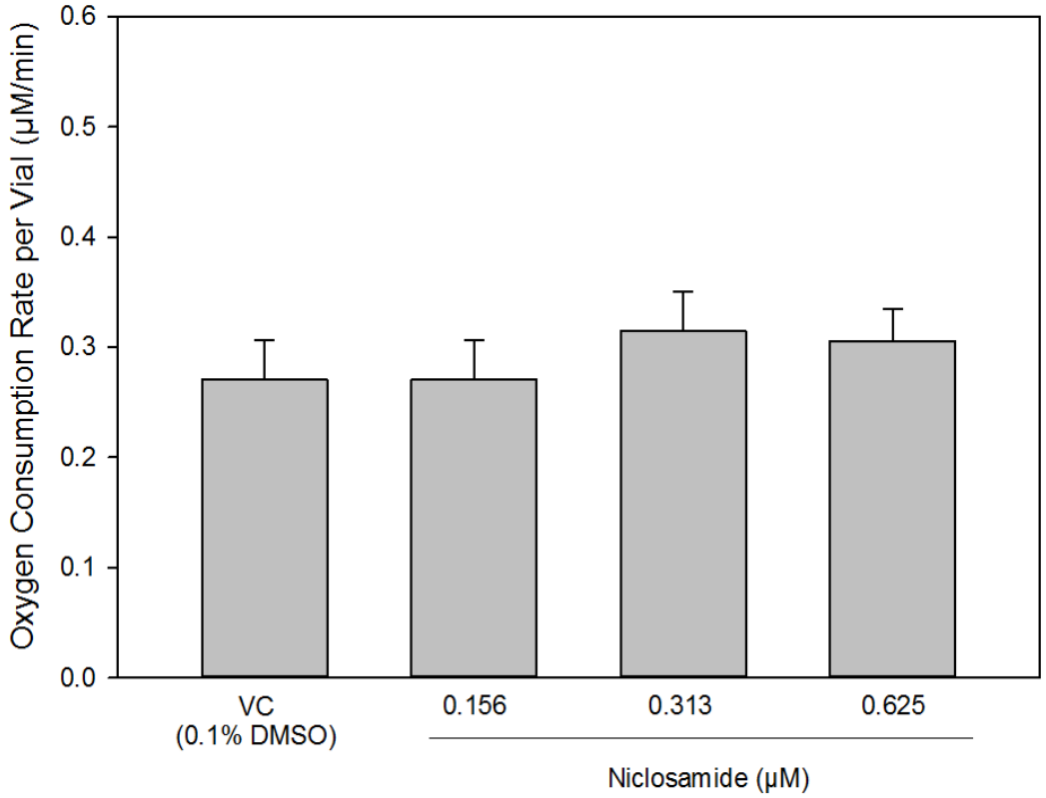
A.E.Bruce, 2016 S. Vliet et al., 2018

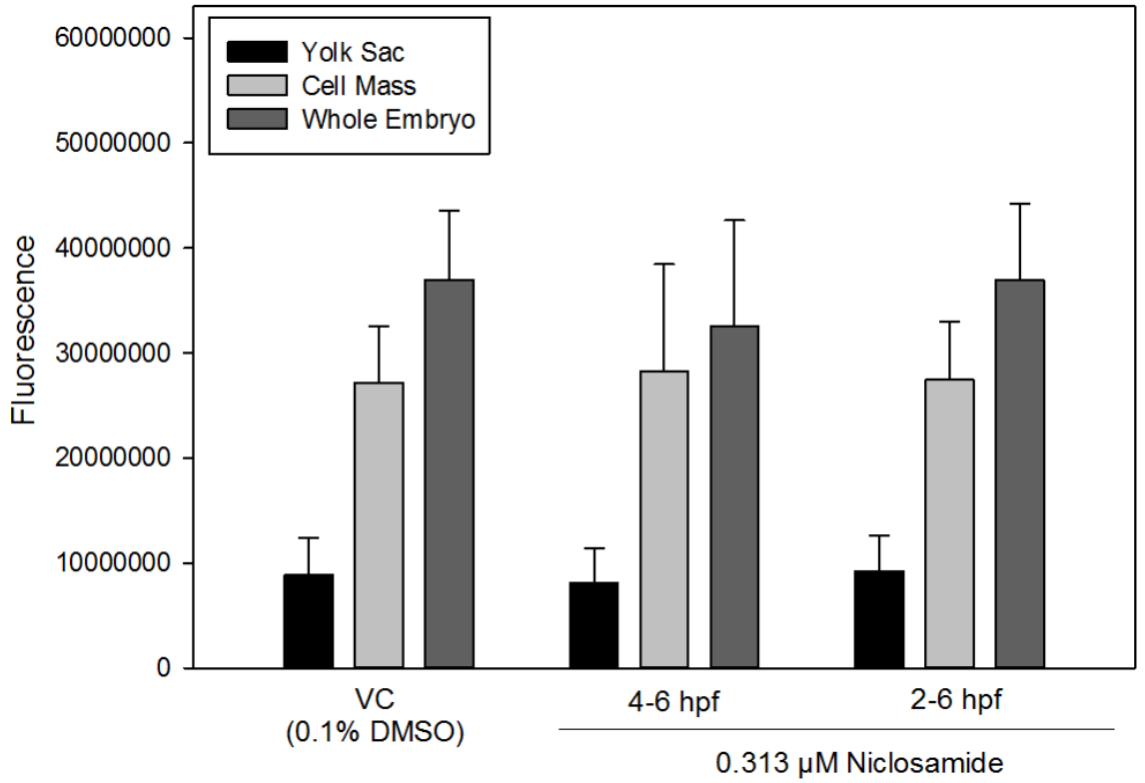
Niclosamide: Let's track down a mechanism

Epiboly delay has been observed in embryos exposed to a variety of compounds with diverse cellular and molecular mechanisms.

- Mitochondrial Impairment
- Alterations to Microtubule Networks
- Zygotic Transcription Inhibition

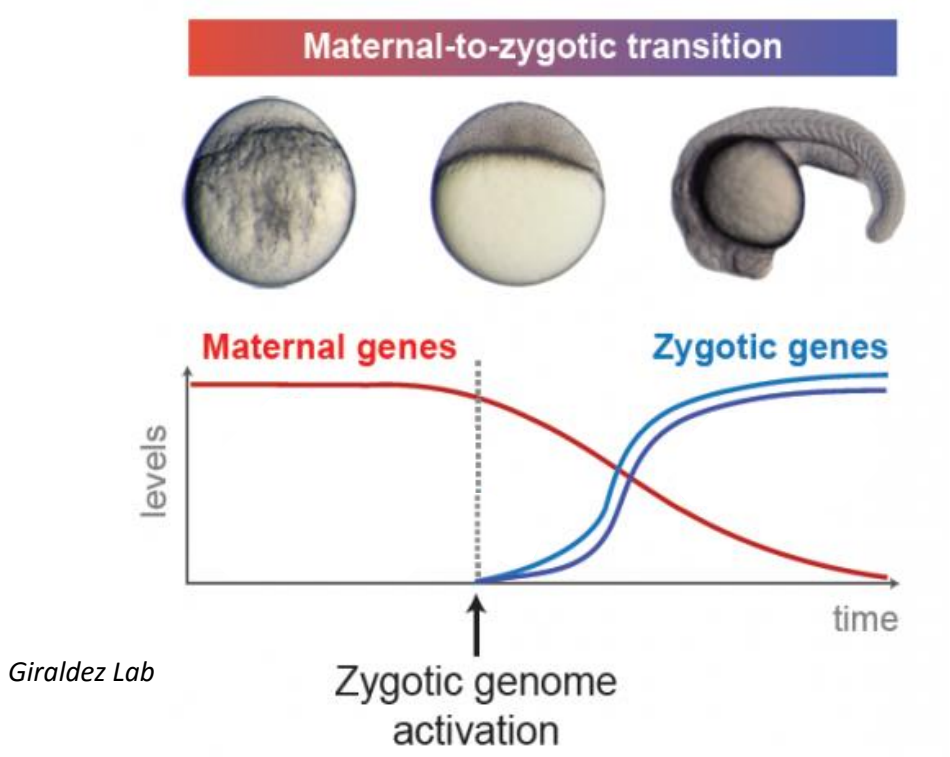






S. Vliet et al., 2018

Let's track down a mechanism: MZT



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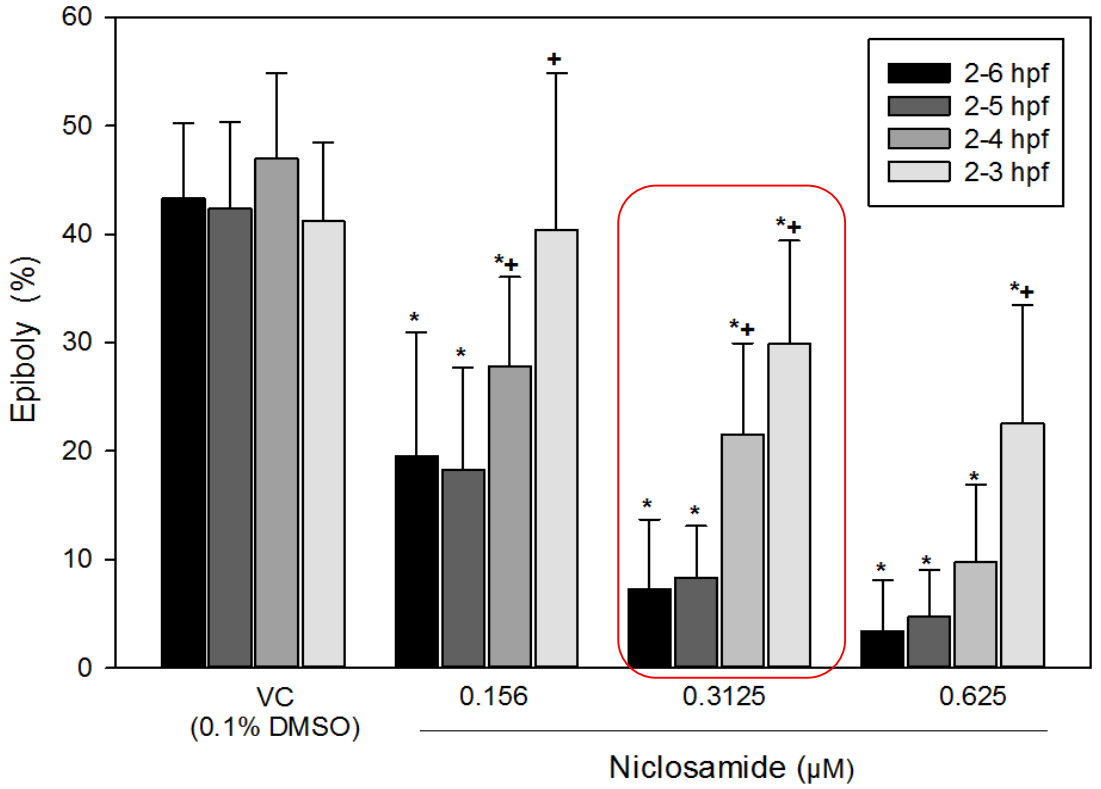
MZT is characterized by the degradation of maternally-deposited mRNA transcripts and the initiation of zygotic mRNA transcription



S. Vliet et al., 2018

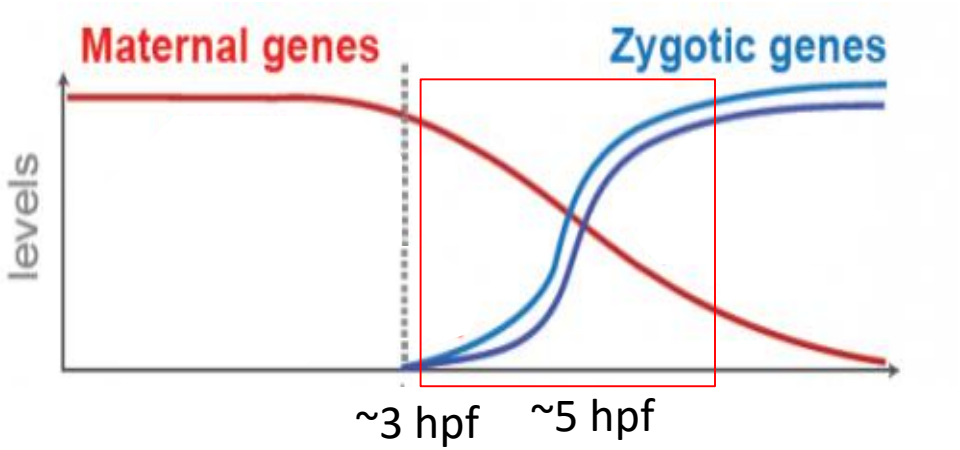
Niclosamide exposure demonstrates a window of sensitivity between 3-4 hpf.

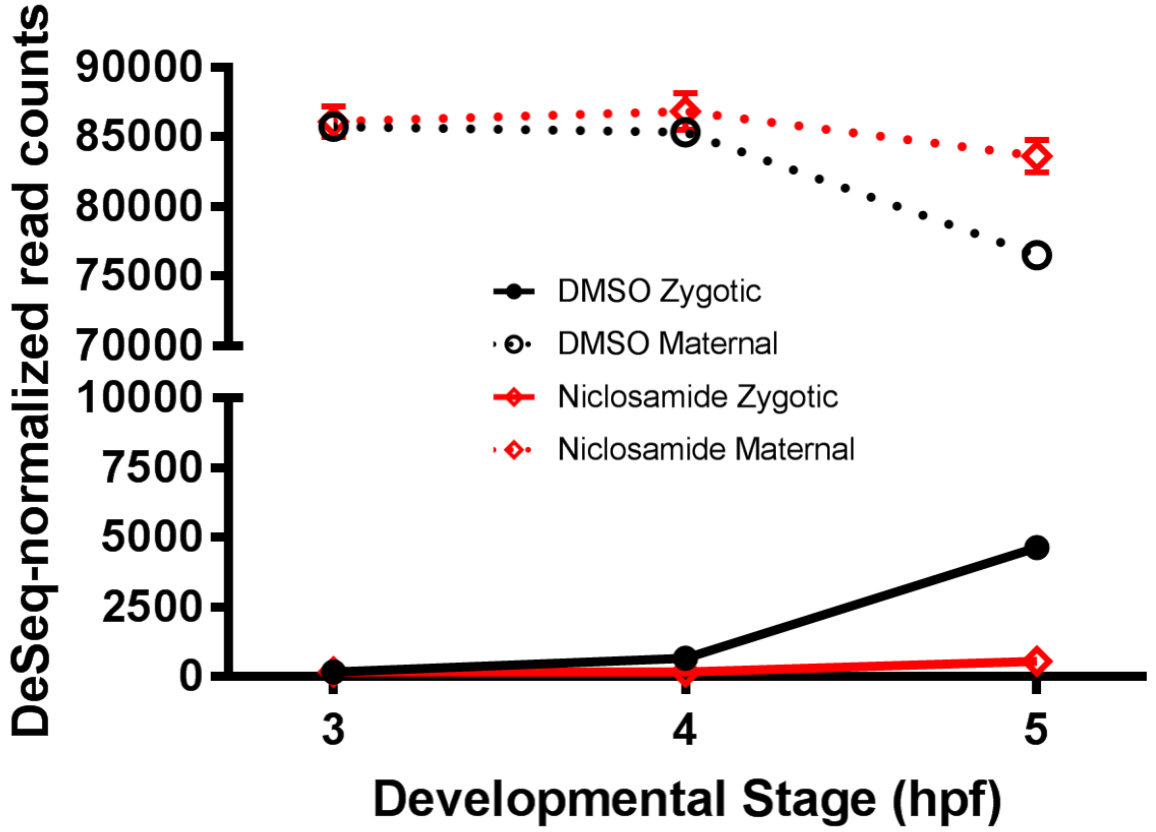
Coincides with the timing of the zebrafish maternal-to-zygotic transition (MZT).

Let's track down a mechanism: MZT

- Niclosamide exposure results in an increased abundance of maternal transcripts and decreased abundance of zygotic transcripts at 5 hpf.

- Niclosamide exposure may cause a delay to maternal transcript degradation and zygotic genome activation.



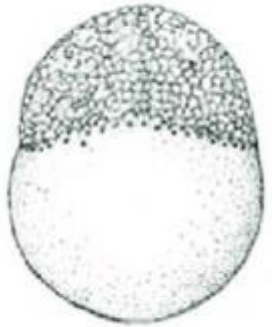


S. Vliet et al., 2018

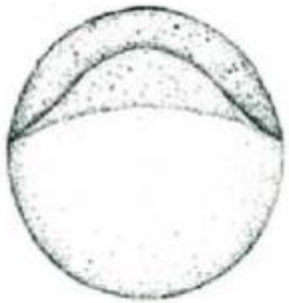
Let's track down a mechanism: mRNA

- Transcriptome of niclosamide-exposed embryos is more similar to stage-matched vs. time-matched
- Impacts to MZT and embryonic development may be due to disruptions to maternal protein translation

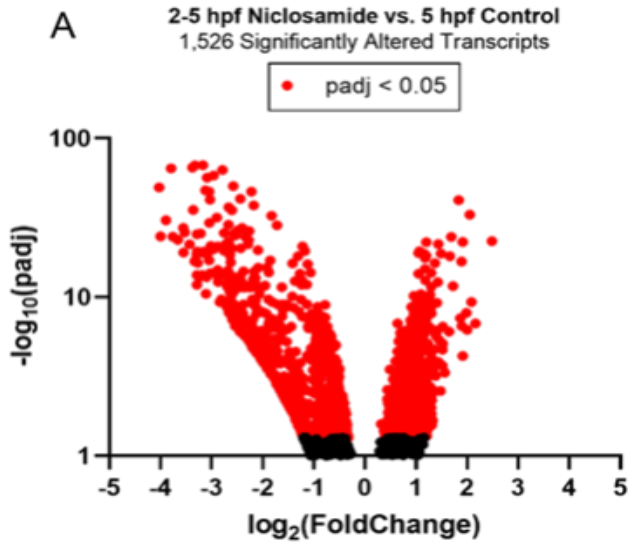
3 hpf

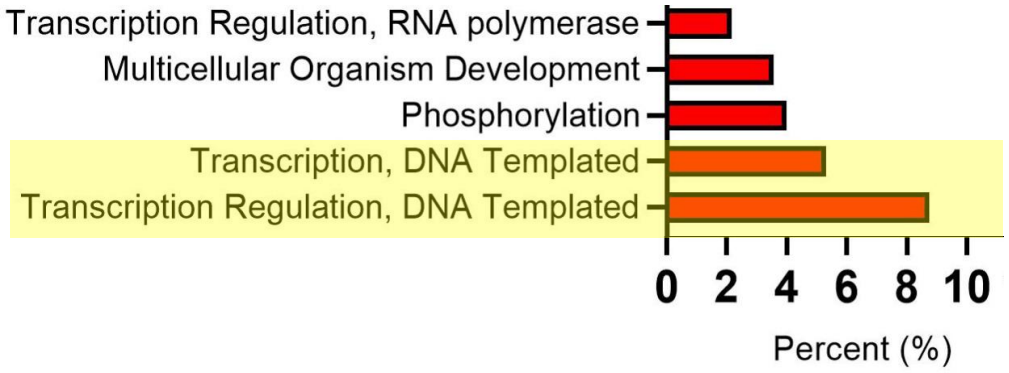


5 hpf

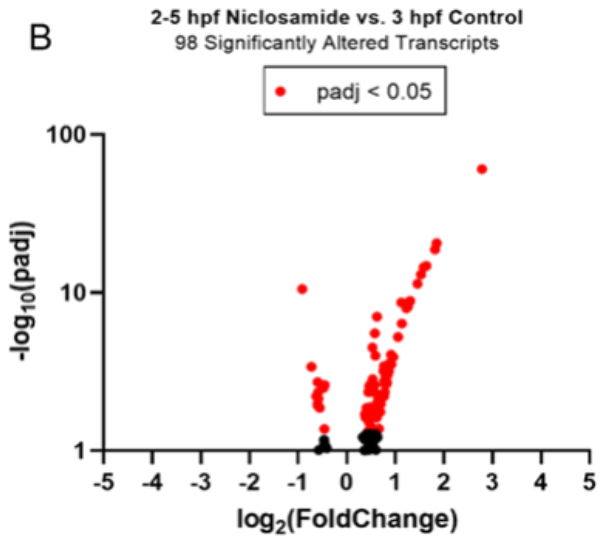


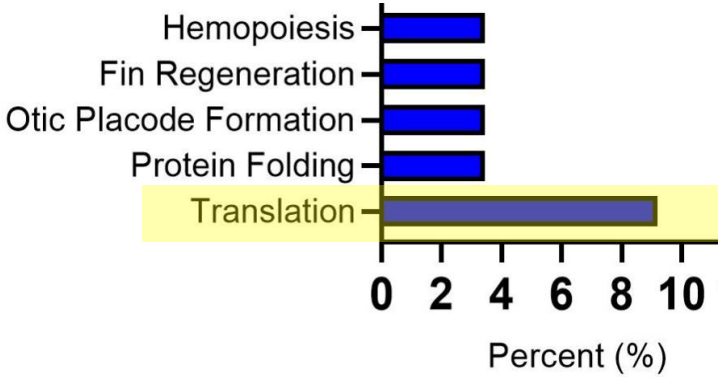
Time-Matched





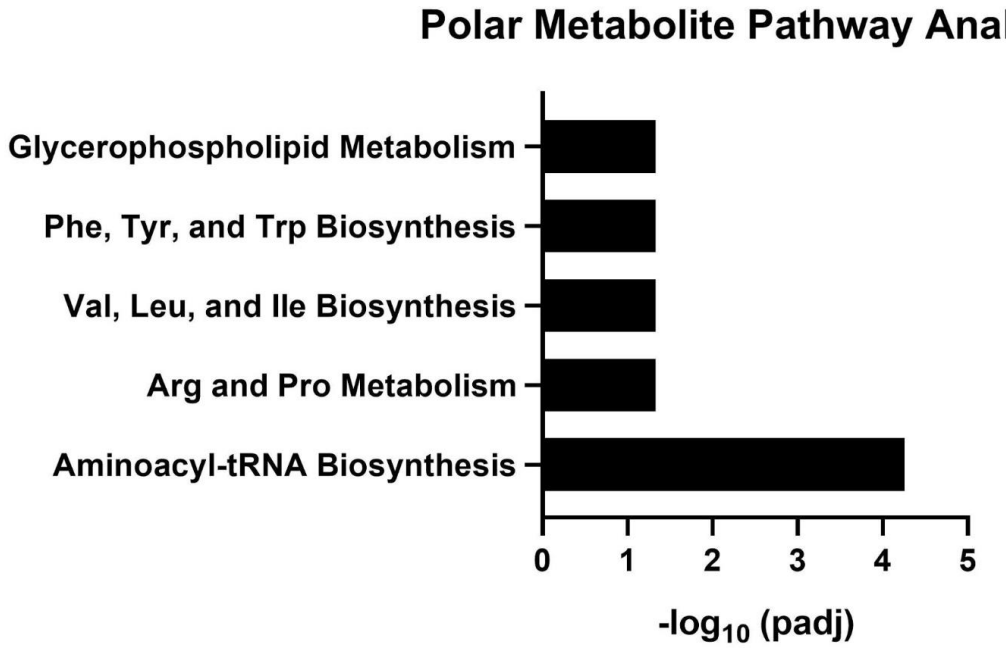
Stage-Matched

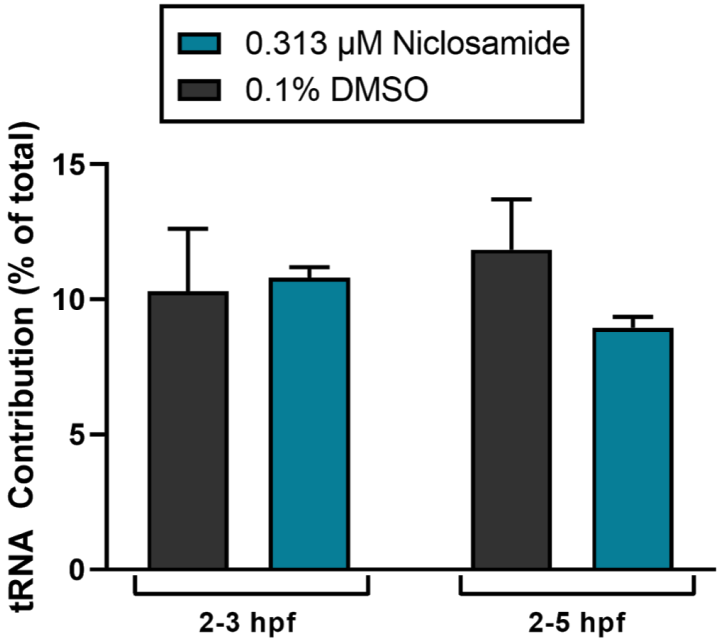




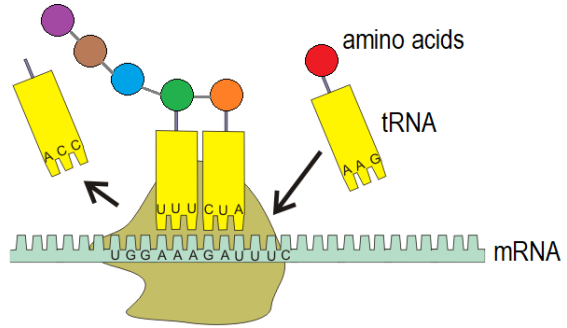
S. Vliet et al., 2019 (In Review)

Let's track down a mechanism: AA-tRNA





- Niclosamide exposure results in significant alterations to non-polar metabolites (Whole-Embryo Metabolomics)
- Altered metabolites associated with AA-tRNA biosynthesis
  - Crucial Process in translation
- Not due to inhibition of tRNA production

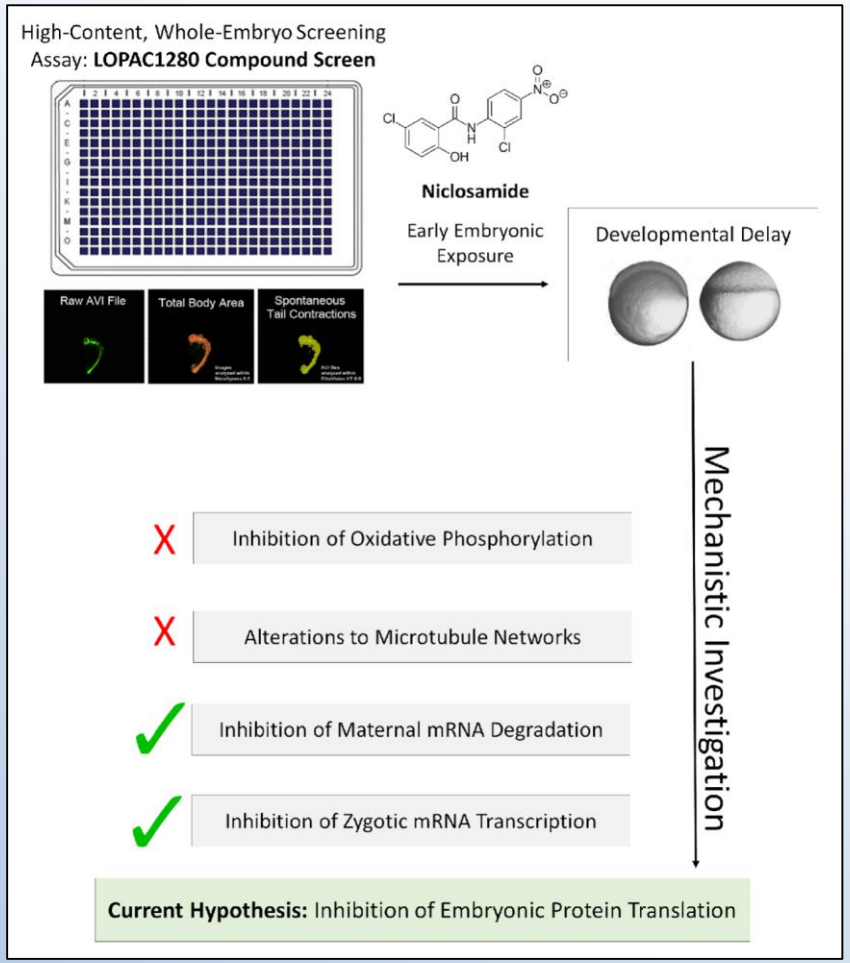


S. Vliet et al., 2019 (In Review)

Conclusions

Overall, our findings highlight the utility of embryonic zebrafish as a physiologically-intact, non-mammalian model for screening of chemicals and environmental samples.

- Demonstrated an unbiased way of progressing from a large dataset to a very specific molecular mechanism.
- Identified an unexpected compound hit with a relevant, and previously unknown mechanism.
- Opened the door for further investigation into compounds disrupting the maternal-to-zygotic transition.



Acknowledgements

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Current Volz Lab Members

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Dr. Subham Dasgupta (Postdoctoral Scholar)
Aalekhya Reddam (PhD Student)
Vanessa Cheng (PhD Student)

Past Volz Lab Members

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Dr. Allison Kupsco
Constance Mitchell, MS
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Questions/Comments?

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