

Boreal Toad (*Anaxyrus boreas*) Microbiome Response to Itraconazole Treatment for *Batrachochytrium dendrobatidis* Infection

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Background

- Boreal toads (*Anaxyrus boreas*) are declining due to the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*).
- The skin microbiome can be a crucial component of amphibian health. Disturbances could change the microbial community to a dysbiotic (unstable) state, increasing susceptibility to infections.
- It is unknown if itraconazole, an anti-fungal treatment for *Bd*, acts as a disturbance to these bacterial communities on *A. boreas*. We aimed to investigate this question during a round of itraconazole treatment.

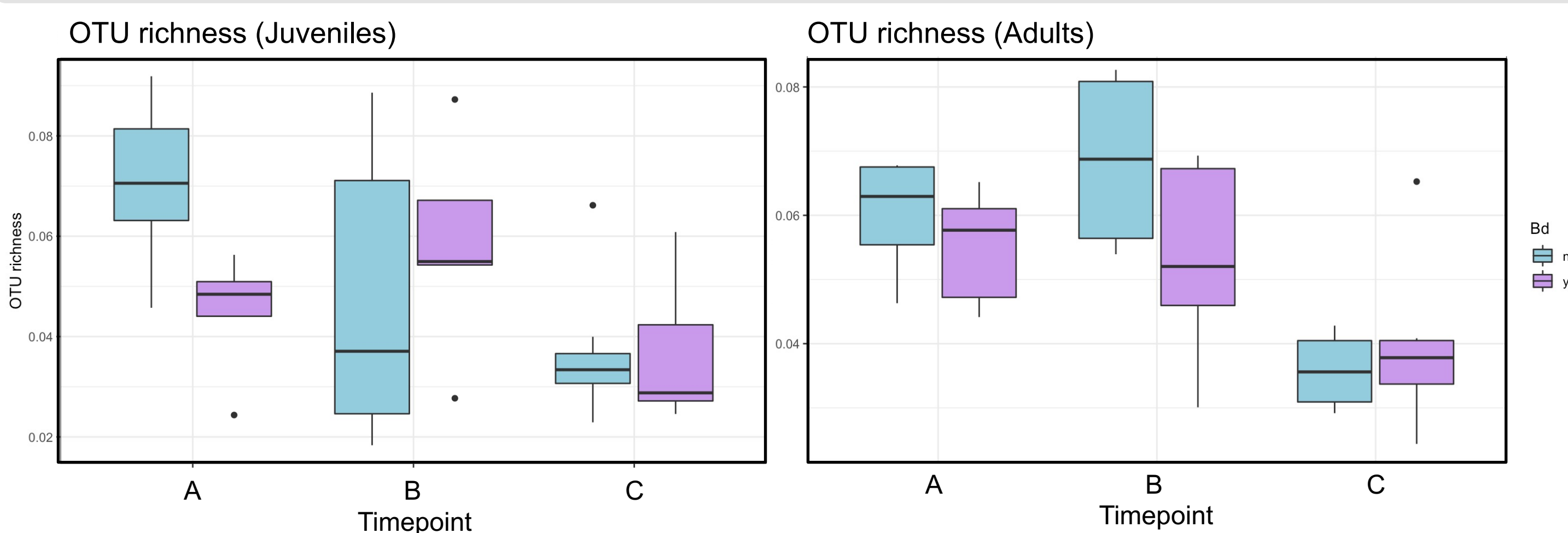


Fig. 1. OTU richness different in juveniles by timepoint ($p=0.007$) and interaction of *Bd* x timepoint ($p=0.020$) but not for initial *Bd* status ($p=0.410$). In adults, timepoint was the only significant factor ($p < 0.001$) but not *Bd* status ($p=0.19$) or the interaction ($p=0.20$).

Methods

- 28 Boreal Toads (16 juveniles; 12 adults) were received for itraconazole treatment from a captive breeding facility after a *Bd* outbreak. It was unknown which individuals were infected.
- Skin swabs were completed before 0.01% itraconazole treatment (timepoint A) and 1 and 2 weeks post treatment (timepoint B and C; respectively).
- DNA extraction and qPCR were used to verify initial *Bd* status. Library preparation of the bacterial 16S region was completed at CMU and sequenced on Illumina MiSeq at University of Colorado Boulder
- Bioinformatics & analysis were completed using Galaxy and the R vegan package.

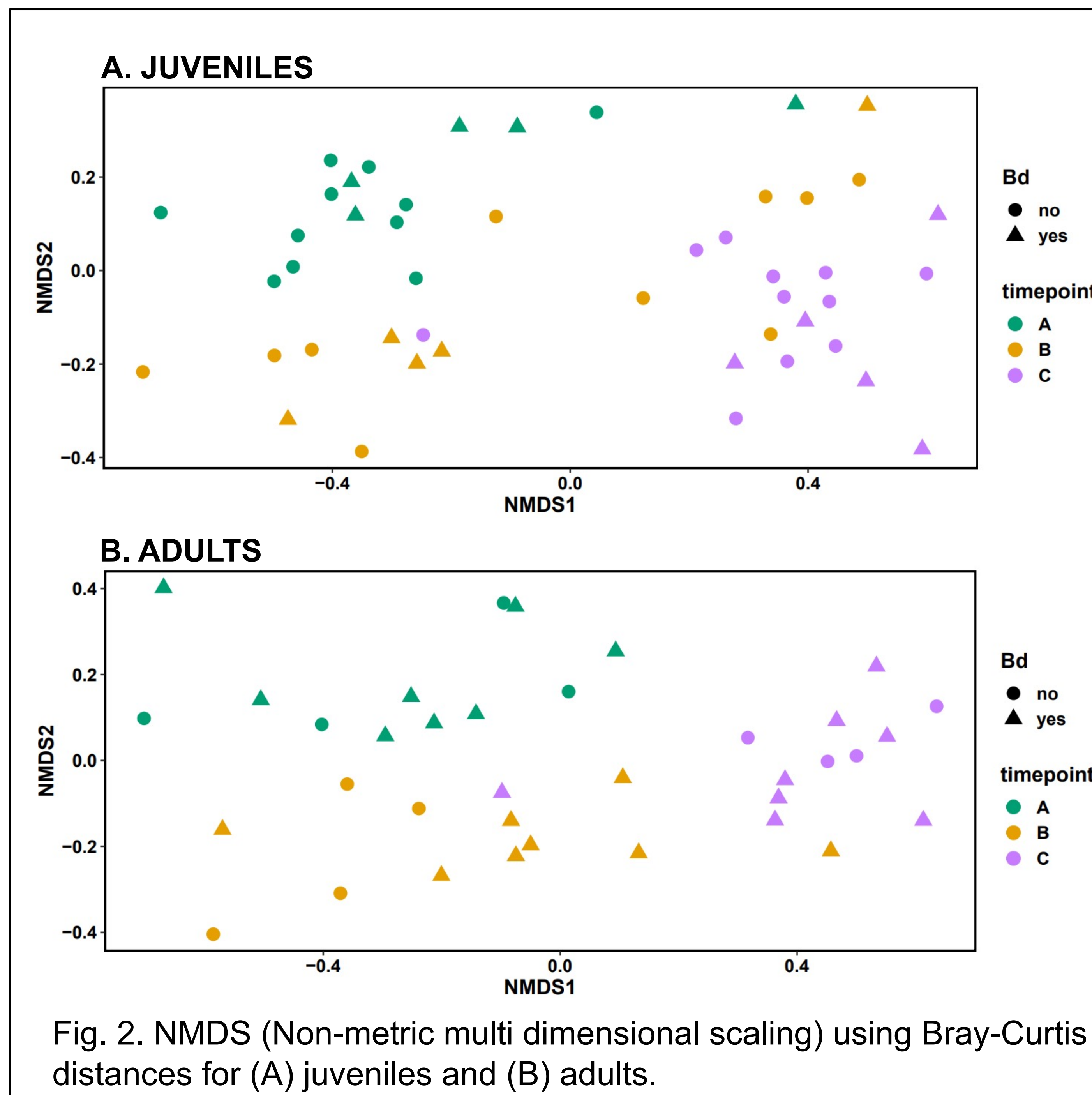


Fig. 2. NMDS (Non-metric multi dimensional scaling) using Bray-Curtis distances for (A) juveniles and (B) adults.

Results

- Preliminary data subset based on 667 OTU's (Operational Taxonomical Units) after quality control.
- RM-ANOVA on Simpson's Diversity Index found timepoint was a significant factor in OTU diversity shifts for both juveniles and adults. *Bd* x timepoint interaction was also significant for juveniles (Fig 1).
- ANOSIM test on Bray-Curtis distances (Fig. 2A & B)
 - Juvenile: *Bd* ($R=0.03$; $p=0.27$); timepoint ($R=0.39$; $p<0.05^*$)
 - Adults: *Bd* ($R=0.057$; $p=1.78$); timepoint ($R=0.50$; $p<0.05^*$)
- 21 Core OTUs (found on 90% of all toads) were identified including 11 found on 100% of toads (Table 1)
- RM-ANOVA (with Bonferroni) on relative abundance of "100% OTUs" found timepoint was a significant factor in many, but *Bd* status prior to treatment did not impact this abundance (Table 1).

Table 1. OTUs found on 100% of all toads at all timepoints. Letter after OTU identity represents lowest taxonomic classification (Class, Order, Family; Genus). Differences in timepoint represented in table by letters for simplicity.

Bacterial OTU	Juveniles	Adults
Comamonadaceae (F)	Timepoint (A-C) ↓	NS
Alphaproteobacteria (C)	Timepoint (A-B)	Timepoint (A-B; B-C)
Gammaproteobacteria (C)	Timepoint (A-C; B-C) ↓	Timepoint (A-C) ↓
Proteobacteria (C)	NS	NS
Hyphomicrobiales (O)	NS	NS
Bacteroidetes (C)	Timepoint (A-B)	NS
Burkholderiales (O)	NS	NS
Sphingobacterium (G)	Timepoint (A-C) ↓	Timepoint (A-B; A-C) ↓
Sphingobacteriales (O)	NS	<i>Bd</i> x Timepoint
Betaproteobacteria (O)	Timepoint (A-B; B-C)	NS

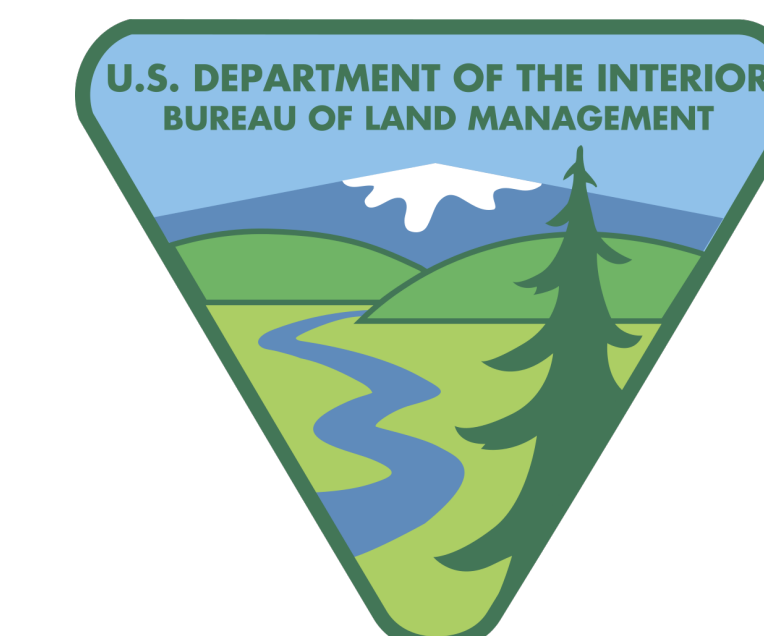
Discussion

- These data suggest that itraconazole impacts community similarity and OTU richness in juvenile and adult *A. boreas* in captivity. These shifts could result in an alternative stable state, recovery, or dysbiosis.
- Later timepoints are needed to better understand if the community recovers to a pre-treatment state.
- Changes to the community after itraconazole treatment in captive breeding facilities may warrant treatment with effective probiotics to restore key community members.
- A limitation to this study is a lack of control individuals that did not undergo itraconazole treatment. We hope to remedy this with a follow-up study.

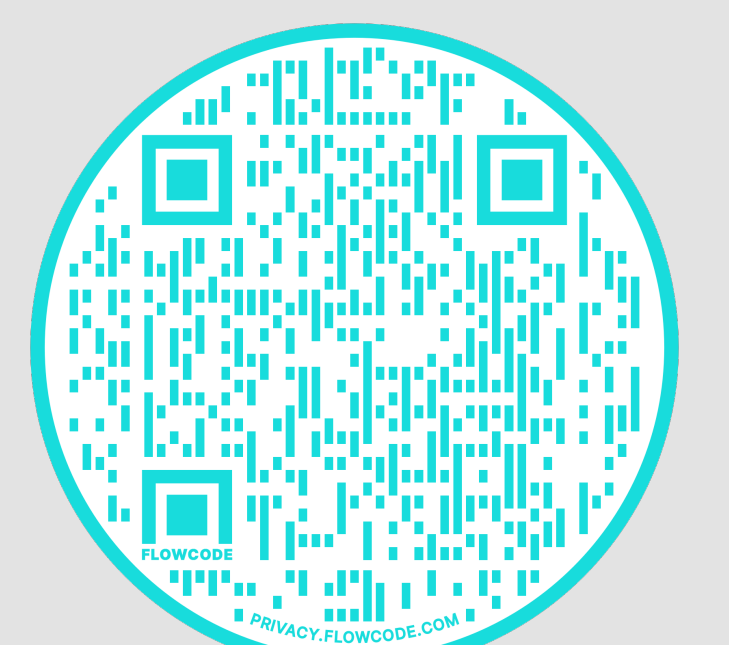
Acknowledgements:



• Faculty Professional Development Funds
• Department of Biological Sciences BioSINQ



References



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