



# The effects of microbial environment and temperature on neurodevelopment in larval amphibians

Kyle Emerson<sup>1</sup>, Samantha Fontaine<sup>2</sup>, Kevin D. Kohl<sup>2</sup>, and Sarah Woodley<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Duquesne University, Pittsburgh PA; <sup>2</sup>University of Pittsburgh

## Introduction

A host and its associated gut microbiota form a symbiotic relationship that plays a major role in host development<sup>1</sup>. In addition, the gut microbiota is part of a bidirectional axis with the brain called the microbiota-gut-brain (MGB) axis<sup>2</sup>. Disruptions to the MGB axis are associated with host neurological impairments and changes in behavior<sup>3</sup>.

Many environmental factors impact the composition and function of the gut microbiome, including temperature and the available microbial species pool. The microbial environment at birth or hatching shapes the gut microbiota which in turn affects host energy acquisition and growth<sup>4</sup>. Further, temperature can alter the diversity and composition of the microbial communities as well as host developmental rate<sup>5,6</sup>. However, it is largely unknown how variation in the local microbial environment and temperature during development impacts neurodevelopment.

Amphibians are an ideal model to study how environmental variation shapes the gut microbiota and host neurodevelopment because they develop completely external to the mother.

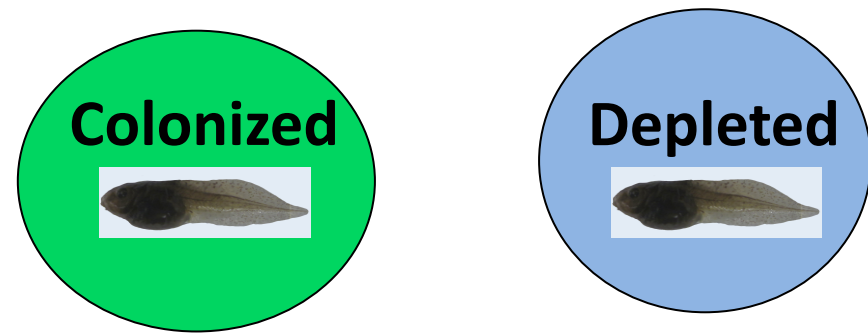
## Hypothesis

**Variation in microbial environment and temperature affects brain size and shape in larval anurans.**

## Predictions

- 1) Development in a **depleted** microbial environment will result in tadpoles with relatively smaller brains and altered brain shape compared to a diverse, **colonized** microbial environment.
- 2) Development in warmer temperatures will result in tadpoles with relatively larger brains and altered brain shape compared to cooler temperatures.

## Microbial Environment

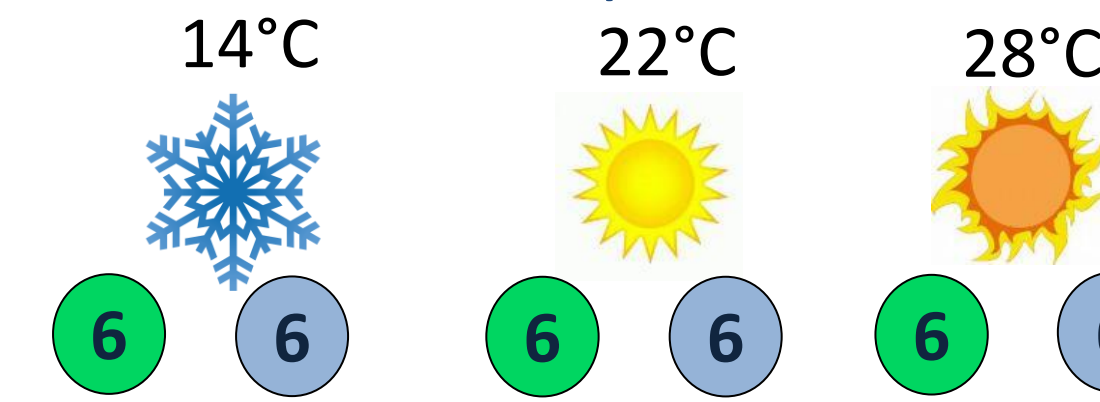


Tadpoles (*Lithobates clamitans*) were raised in 2 microbial environments for 7 weeks at 22°C:

- **Colonized**: 25% natural pond water; 75% autoclaved tap water.
- **Depleted**: 25% autoclaved pond water; 75% autoclaved tap water.

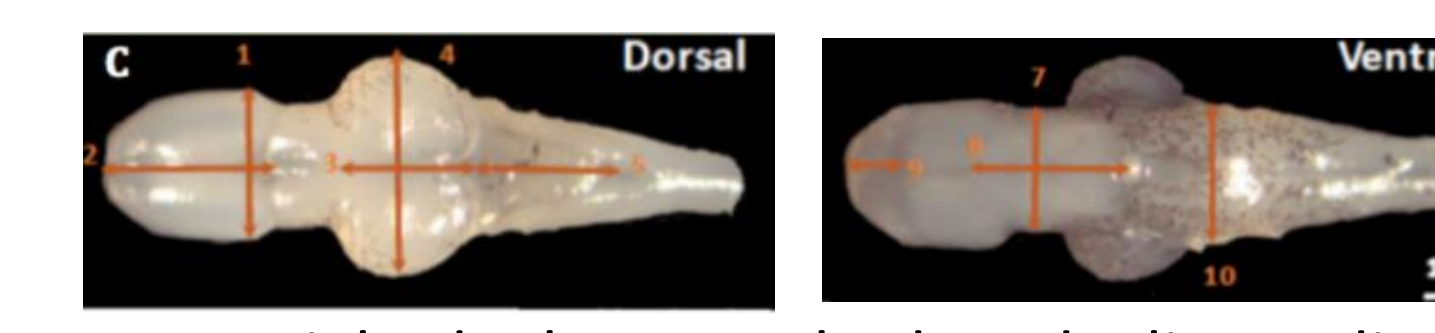
## Methods

### Temperature



- After 7 weeks, tadpoles were exposed to different temperatures for 3 weeks: 14°C, 22°C, 28°C. Microbial environment was maintained during this time.
- Had 6 replicates (each with 5 tadpoles) per treatment combination.

### Brain Morphometrics



- Brains were weighed, photographed, and 7 linear dimensions were measured using Image J.
- To correct for body mass, brain mass was expressed as unstandardized residuals from linear regression.
- Linear brain dimensions were mass adjusted and reduced to 2 principal components. Principal components were analyzed with 2-way ANOVA.

## Conclusions

- Both microbial environment and temperature resulted in changes in the gut microbiota composition (data not shown, Fontaine and Kohl). Thus, the effects of the microbial environment and temperature on brain development could be due to impacts on the gut microbiota.
- As predicted, development in warmer temperatures increased relative brain mass and altered relative brain shape. This is likely due to the role of temperature in ectothermic digestion and energy assimilation<sup>5</sup>.

- As predicted, development in a microbially diverse environment resulted in a relatively larger brain, although there was no change in brain shape.

## Future Directions

- Determine the functional consequences of the impacts on brain mass and shape by measuring visual and olfactory behavior and endocrine function.
- Correlate specific changes in brain mass and shape to gut microbial profiles in anurans.

## Take Home Message

**Variation in the microbial environment and temperature during development contributes to brain plasticity.**

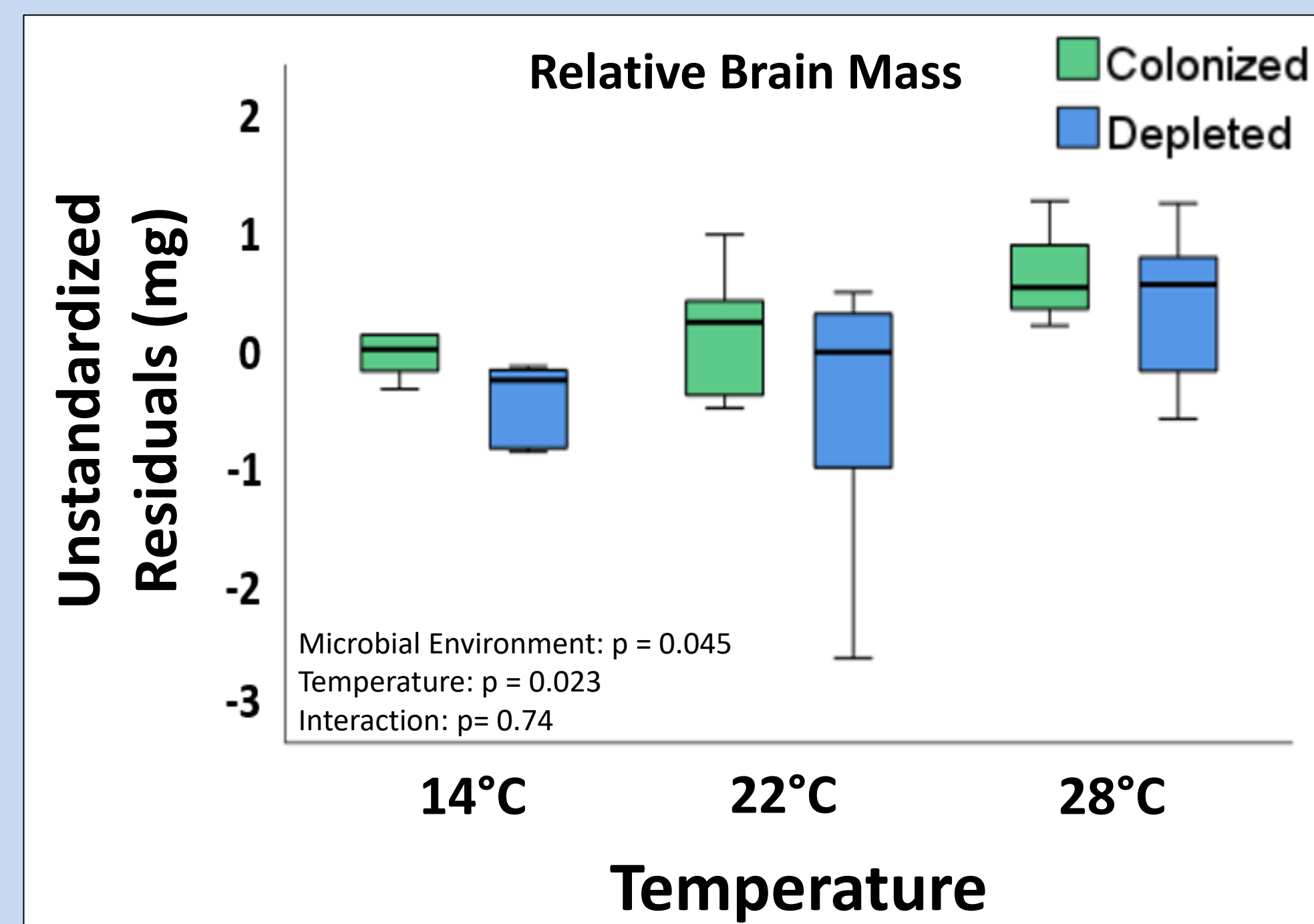
## Acknowledgments

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## Citations

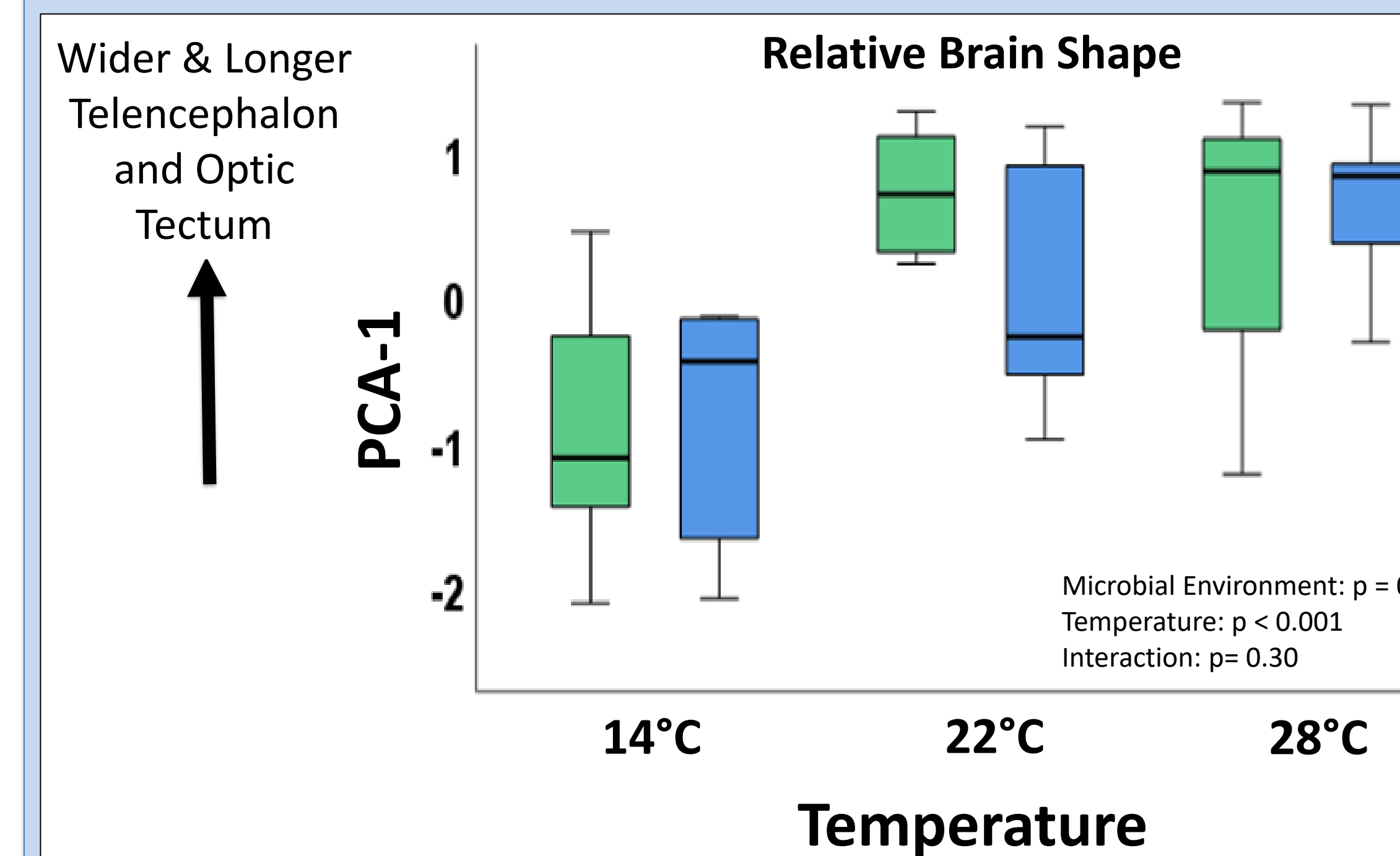
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## Results



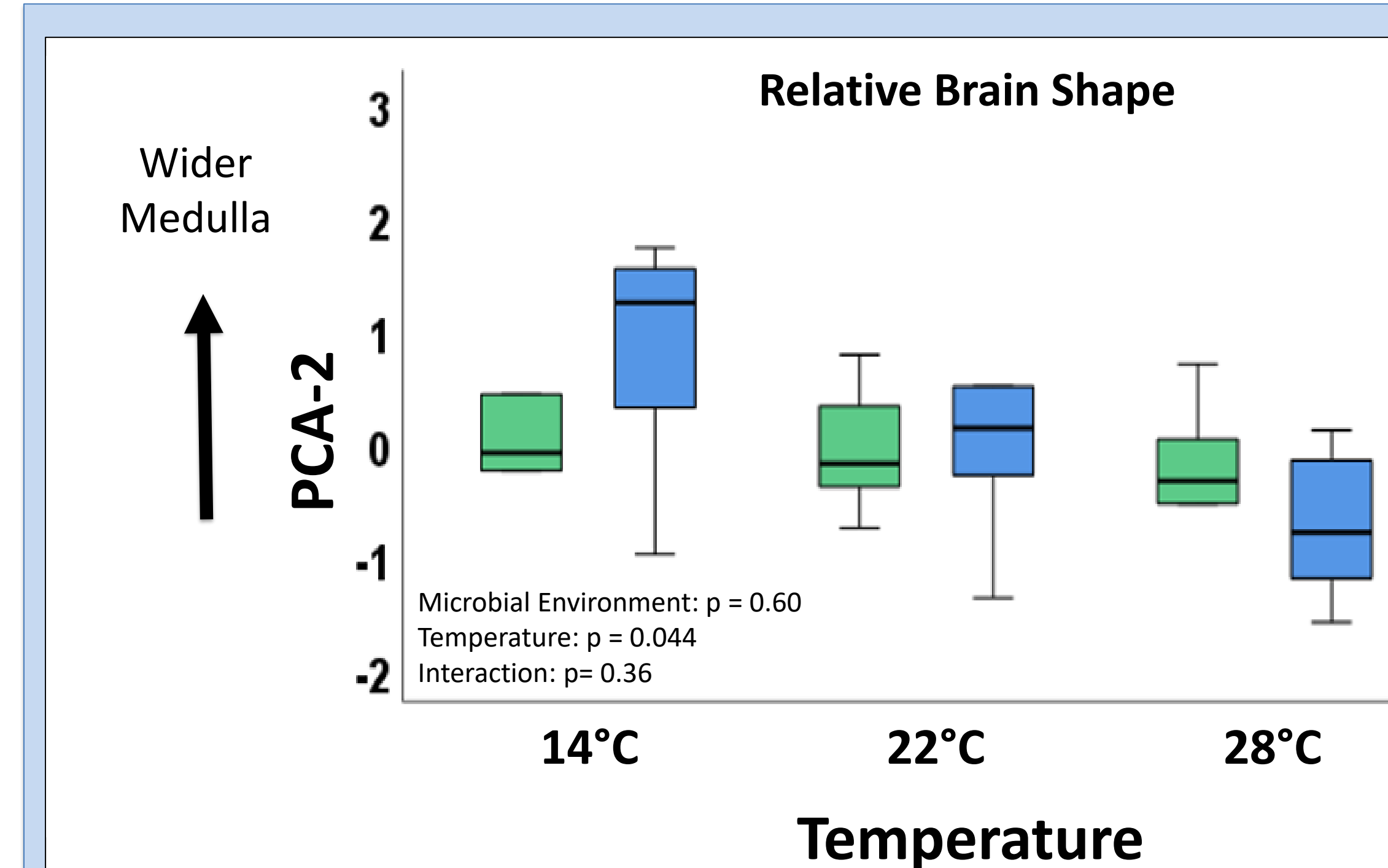
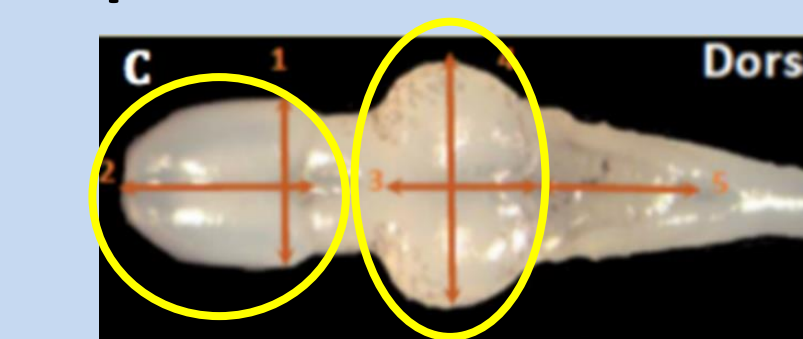
**Figure 1:** Relative brain mass of tadpoles raised in microbially depleted or colonized environments and different temperatures.

**Takeaway 1:** Development in a **depleted** environment resulted in tadpoles with relatively smaller brains. Development in warmer temperatures resulted in tadpoles with relatively larger brains.



**Figure 2:** Relative shape of the telencephalon and optic tectum of tadpoles raised in microbially depleted or colonized environments and different temperatures.

**Takeaway 2:** Warmer temperatures resulted in tadpoles with relatively wider and longer telencephalons and optic tecta. Microbial environment had no impact on telencephalon or optic tectum shape.



**Figure 3:** Relative shape of the medulla of tadpoles raised in microbially depleted or colonized environments and different temperatures.

**Takeaway 3:** Warmer temperatures resulted in tadpoles with relatively narrower medullas. Microbial environment had no impact on medulla width.

