The Assembly of Microbial Communities on Wood Frog (*Rana sylvatica*) Tadpoles: The Influence of the Environment

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INTRODUCTION

From very early in development, the microbiome is involved in processes critical to its host, including nutrient acquisition, immune system modulation, and protection against infections. Few studies have investigated how environmental conditions alter the early formation of the microbiome. In this study, we determined if the environment wood frog tadpoles (*Rana sylvatica*) are reared in affects formation of the bacterial communities of the gut and skin. Further, we assessed how each treatment altered the overall growth and development of the tadpoles. Theory predicts that functionality should increase with the diversity of the host symbiont community. We, therefore, hypothesized the environment with pond water and pond soil would result in the most diverse microbiomes, and that tadpoles in this treatment would be larger and reach metamorphosis more quickly as a result.

A wood frog tadpole

METHODS

We collected newly laid egg masses, water, and soil from one pond on the Vassar College campus.
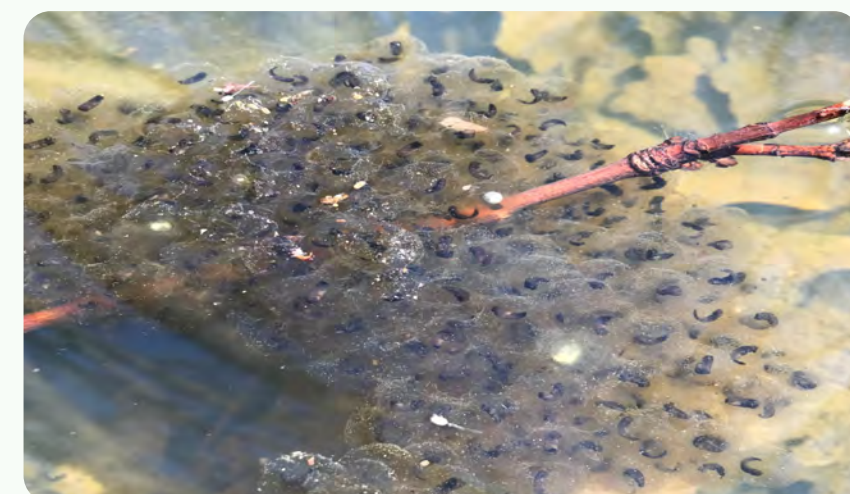
Eggs were randomly sorted into treatments:
- Artificial water (AW), N=10
- Pond water (PW), N=10
- Pond water + soil (PWS), N=10
- Sterilized pond water (SP), N=10
- Sterilized pond water + soil (SPS), N=10

Tadpoles were reared individually in the lab until they reached Gosner Stage 38-42. Water and soil were changed weekly, and they were fed bi-weekly.

Dissections were performed and DNA was extracted from gut and tail tissues. The 16s rRNA gene was sequenced to characterize the bacterial communities present.

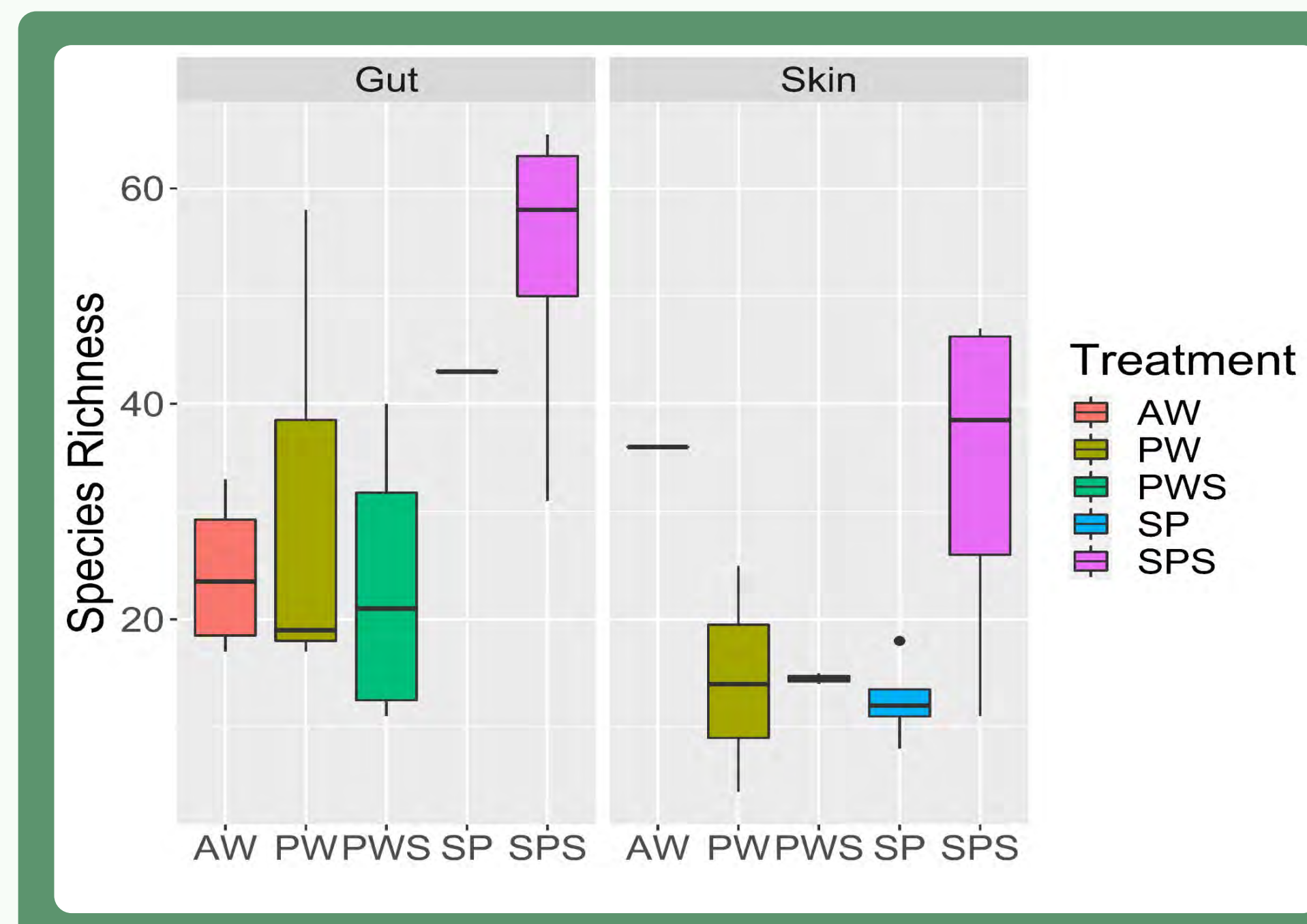Sequencing data were analyzed using QIIME2 and R.

RESULTS

Figure 1. Species richness differed across environments for the gut (ANOVA: $F_{4,9}$=3.28, P=0.05), but not for the skin (ANOVA: $F_{4,9}$=2.77, P=0.09) . Tadpoles reared in sterile pond water with soil (SPS) had more diverse gut bacterial communities than those from other treatments.
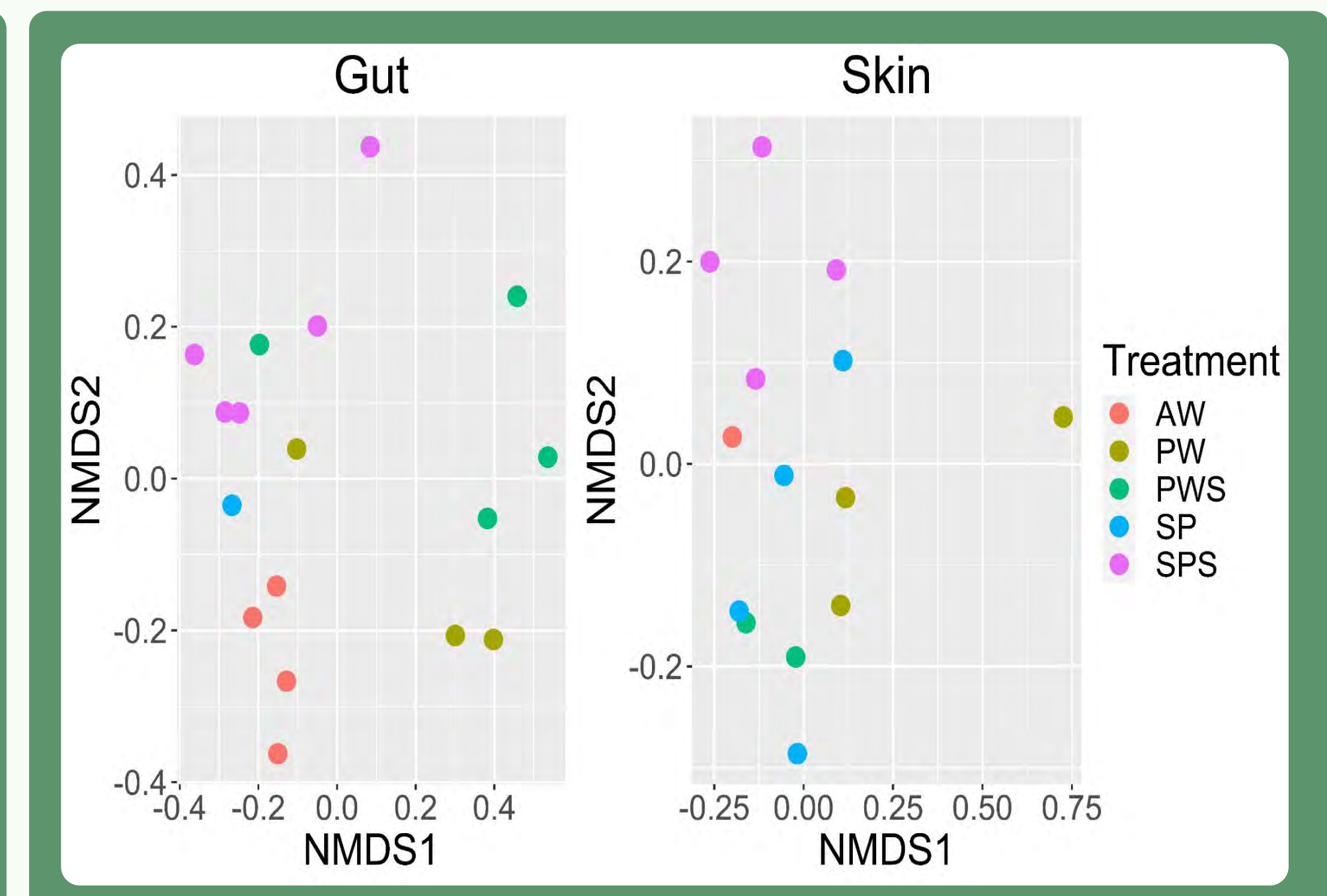

Figure 2. Rearing environment influenced bacterial species composition in the gut (PERMANOVA: R2=0.39, P=0.002) and on the skin (PERMANOVA: R2=0.40, P=0.016) of tadpoles.
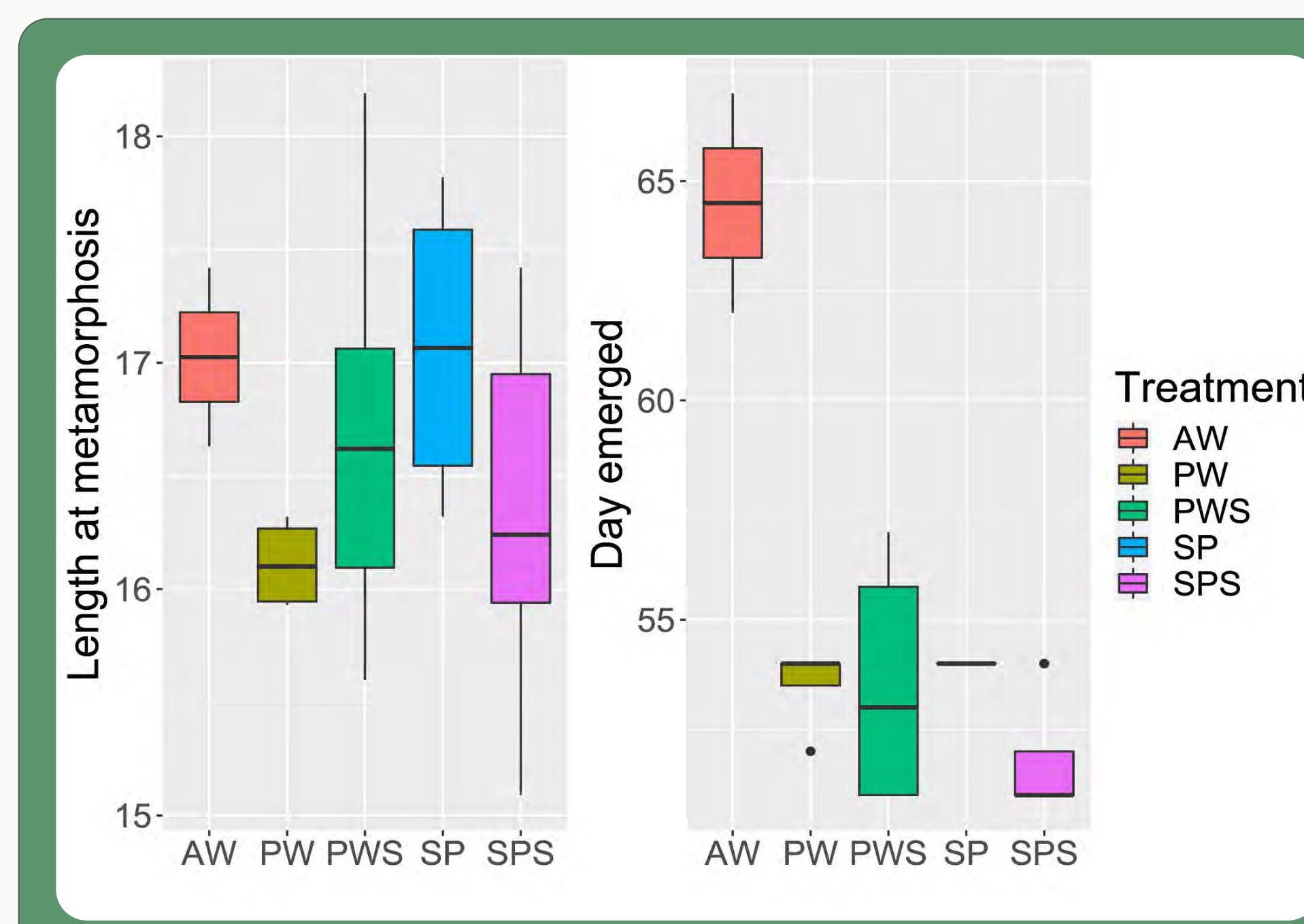

Figure 3. Tadpoles from all environments grew to a similar size (ANOVA: $F_{4,20}$=1.73, P=0.18), but tadpoles reared in artificial water (AW) took longer to reach metamorphosis than those from other treatments (ANOVA: $F_{4,20}$=19.46, P<0.001).

CONCLUSIONS

- Early formation of the tadpole microbiome was influenced by the surrounding environment, affecting both the number and kinds of bacterial species present in the skin and the gut. This may be due to the direct influence of environmental microbes colonizing the host.
- Sterile pond water with soil resulted in the most diverse gut bacterial community, perhaps a result of soil microbes leaching into the water and inhabiting tadpoles.
- The environment also influenced time to metamorphosis. Tadpoles reared in artificial pond water may lack access to critical nutrients necessary to develop normally.

ACKNOWLEDGEMENTS

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