

Dear Editors

My manuscript "**Comparison of the composition and function of gut microbes between adult and juvenile *Cipangopaludina chinensis* in the rice snail system**" has been revised according to your guidance. We thank the reviewers for their generous comments on the manuscript and have have edited the manuscript to address their concerns. We hope that they will be satisfied.

Under your guidance, we believe that the manuscript is now suitable for publication in PeerJ.

Sincerely yours

Mr. Xianhui Pan

On behalf of all authors

Reviewer 3 (Anonymous)

Basic reporting

Ok

Reply: Thanks for the affirmation of the reviewers.

Experimental design

In the rebuttal the authors said "Moreover, we extracted DNA for each parallel group, and then extracted an equal amount of DNA samples from the 10 individuals in the parallel group and pooled them for library construction, in order to avoid the influence of individual differences."

My previous review was to reject the paper based on the sample size obscurity. Now that is clarified. But if you examine the initial study objective as stated in the intro:

"Herein, it is necessary to systematically understand the dynamic 67 changes of intestinal microflora of *C. chinensis* so as to develop the best diet for snails. "

"In this study, we performed high-71 throughput sequencing of 16S rRNA gene to study the function and composition of intestinal microbiota in

72 adult and juvenile *C. chinensis* under artificial habitat. This result provides insight into the reasons for

73 differences in feeding behavior and food preferences between juvenile and adult snails. "

In my view, individual responses would have been necessary to achieve this goal. If it were just a survey of microbiome compositional differences, I presume pooling would be fine. But given the initial objective, my opinion remains the same. The study is valid, and the other changes are all acceptable and great. I just think that as the objectives are stated it cannot be accepted. Now if this is published a survey of microbiome differences as a hypothesis-generating study, then my opinion would be to accept it.

Reply: Thank you for your professional and constructive comments. The purpose of this study was to preliminarily explore the differences in gut microbial communities in different growth stages of *C. chinensis* (adult and juvenile stage). We have revised and refined lines 72-73 and 80-82 in the manuscript based on the comments of the reviewers, which hopefully meet the reviewers' requirements.