Cover letter:

Dear Editor,

I am writing on behalf of my co-authors to submit our manuscript titled "Characterizing the secret diets of siphonophores (Cnidaria:Hydrozoa) using DNA metabarcoding" to be considered for publication in PLOS One. All authors approve of the manuscript and its submission to PLOS One. The manuscript is original work and has not been simultaneously submitted to any other journal.

We believe that this manuscript will be of interest to a broad readership and is appropriate for publication in PLOS One, as it advances our understanding of marine trophic ecology and pelagic food web structure, and constitutes the first application of DNA metabarcoding to elucidate the diets of oceanic gelatinous predators. The structure of oceanic food webs is critical for the sustainability of marine ecosystems, commercial fisheries, and the biological carbon pump. Thus, it is essential to understand its structure and how changing the community composition will affect its architecture. This knowledge will allow scientists to better predict the impacts of climate change, overfishing, and conservation policies on the functioning of oceanic ecosystems. This work will draw attention from scientists in the fields of food web ecology, biological oceanography, and cnidarian zoology.

In this study, we apply DNA metabarcoding to reveal the diets of siphonophores, a group of understudied open-ocean gelatinous predators. In open-ocean food webs, gelatinous zooplankton constitute one of the most abundant and diverse animal groups, occupying central positions in the food web as predators and prey of each other. This gelatinous fraction is often labeled the 'jelly-web' (https://doi.org/10.1002/lno.11605), and comprises an understudied but large part of the trophic connectivity in these ecosystems. Our results provide the first insight into the diets of nine siphonophore species and compare our findings in another fifteen species with those from previous visual gut content inspections and remotely-operated vehicle observations across different pelagic depth habitats. We also use these results to evaluate the dietary predictions posited in Damian-Serrano et al. 2021 (https://doi.org/10.1093/iob/obab019) based on tentacle and stinging-capsule morphologies.

Our analyses detected both small and gelatinous prey taxa underrepresented by visual methods in species from both shallow and deep habitats, indicating that siphonophores play similar trophic roles across depth habitats. We also reveal hidden links between siphonophores and gelatinous filter-feeders such as salps and larvaceans, which transfer nutrients from herbivorous phytoplankton consumers to higher trophic levels in the food-web. Our results suggest a greater involvement of siphonophores in the 'jelly-web' than previously considered. These findings also confirm suspected biases against rapidly-digested gelatinous prey in visual gut-content inspection which had precluded a comprehensive understanding of the role of these animals in the food web. We also find a large prevalence of small prey in deep sea species which may have gone unnoticed in submersible observations, as suggested in (https://doi.org/10.1098/rspb.2017.2116). Finally, we found support for the predicted diets for seven siphonophore species, validating the eco-morphological link between tentacle morphology and diet.

Collecting these new data from siphonophore gut contents was a major undertaking. Siphonophores are fragile animals that live far offshore and in the deep sea, and thus are extremely hard to collect. Specimen collection required SCUBA diving and the deployment of remotely operated vehicles hundreds of kilometers from shore. We developed and optimized a custom metabarcoding protocol with six different barcode regions. In addition, we collected, identified, and sequenced several underrepresented pelagic species in order to enhance the accuracy of our taxonomic assignments.

Thank you for your consideration of our manuscript.

Yours sincerely,

Alejandro Damian-Serrano, Ph.D.