Unravelling the dinner menu: First comparative study on natural food composition of juvenile *Scylla olivacea* and *Scylla tranquebarica* with Oxford Nanopore Technology sequencing reveals trophic niche difference

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As of 2021, South Sulawesi province has converted 119,191 hectares of secondary mangrove areas into aquaculture ponds for commercial shrimp (Penaeus spp.) and mud crab (Scylla spp.), making it the largest province in Indonesia in terms of mangrove conversion. Indonesia plans to expand mangrove cover by transitioning from conventional aquaculture to sustainable practices to address carbon emissions and align with the Forestry and Other Land Uses (FOLU) net sink 2030 mandate. The Ecosystem Approach to Aquaculture (EAA) offers a promising opportunity to balance aquaculture productivity and mangrove conservation. However, one critical knowledge gap is the feasibility of polyculture for *Scylla* spp., particularly concerning trophic niche overlap during juvenile stages. This study examined whether juvenile *Scylla olivacea* and *S. tranquebarica* share trophic components, which would influence co-stocking strategies.

We conducted the research in Sungai Ujung, Maros, South Sulawesi, a key aquaculture hub. During active feeding periods in August 2023, we collected a) juvenile male mud crabs (inner carapace width < 80 mm) using locally crafted spear-like bamboo tools, and b) salinity data using a hand-salino-refractometer. Fecal samples from 7 individuals were analysed using Next-Generation Sequencing (NGS) via Oxford Nanopore Technology (ONT) with the Folmer primers (mtCO1 region, 709 bp) as markers. We conducted library preparations using the SQK-LSK 114 kit from ONT. Nanopore sequencing was operated with MinKNOW software v. 23.07.12. We performed downstream analysis and visualisations using Pavian, Krona Tools, and RStudio with R v. 4.2.0.

The analysis identified two *Scylla* species: five individuals of *S. olivacea* and two of *S. tranquebarica*. Key findings include the following: 1) The ONT read compositions are as follow. For S. olivacea, ONT reads consisted of 87,481% potentially host DNA, 6,777% DNA from other *Scylla* species, and 5,741% prey DNA. For *S. tranquebarica*, ONT reads consisted of 89,466% potentially host DNA, 9,745% DNA from other Scylla species, and 0,784% prey DNA. 2) We identified a total of 952 genera across all samples. Of these, we classified 137 genera (including genus *Scylla*) with more than ten copies (reads) as the prey species directly ingested by the crabs. We assumed the remaining genera originated from the crab's consumed prey rather than direct predation. 3) Trophic niche differences are as follow. A) *S. olivacea* and *S. tranquebarica* shared 11 of the 136 prey genera. However, distinct prey preferences emerged: *S. tranquebarica* consumed exclusive prey from 18 genera, while *S. olivacea* showed broader prey diversity (exclusively preyed on 106 genera). B) Dominant prey genera for *S. olivacea* included mangrove swimming crabs (Charybdis), freshwater algae (Thorea), and plant parasitic butterflies (Battus). In contrast, *S. tranquebarica* primarily consumed brackish water mussels (Parabrachidontes and Mytella), and mangrove swimming crabs (Charybdis). The Mann-Whitney U test revealed a significant difference (W = 15664, p-value: 32.2^{e-16}) in prey composition between the two groups. 4) Salinity levels ranged from 10.17 to 21.33 psu, confirming suitable habitat conditions for both *S. olivacea* and *S. tranquebarica*, which are known to tolerate salinities between 16 and 32 psu. The presence of both *S. olivacea* and *S. tranquebarica* within the same mangrove area demonstrates habitat co-existence but reveals significant trophic niche divergence. However, the cannibalism hints found in *S. tranquebarica* on *S. olivacea*, indicate that the polyculture grow-out system might not be feasible.

These findings contribute critical data for designing EAA-based mangrove pens in Indonesia. However, to strengthen these conclusions and ensure the sustainability of aquaculture practices, further studies should incorporate larger sample sizes and different temporal sampling regime. By addressing these knowledge gaps, aquaculture practices can align more effectively with Indonesia's FOLU net sink 2030 mandate and its EAA-based aquaculture development.

Keywords
Ecosystem services in aquaculture; Juvenile feeding ecology; Genomic data analysis