Final Report

Brilliant Marine Research Idea 2025

This report presents the results of research conducted under the Brilliant Marine Research Idea grant, supported by The Sea as a Good Cause (VLIZ philanthropy).

1. General information

Title of the idea	Cracking the trophic code: Integrating DNA metabarcoding and isotope analysis to implement mud crabs *Scylla* spp. sustainable aquaculture
Name PhD student	Arida Fauziyah
Name supervisor	Prof. Marc Kochzius
Flemish University or University College	Vrije Universiteit Brussel

2. Brilliant Marine Research Idea – Report about the activities

Abstract

Conventional grow-out pond systems for mud crabs (*Scylla* spp.) have supported aquaculture production in Indonesia but contributed to extensive mangrove degradation and inconsistent culture performance. Co-stocking of morphologically similar species often results in variable growth and survival. These practices conflict with Indonesia's FOLU Net Sink 2030 commitment to halt mangrove deforestation and enhance carbon sequestration. South Sulawesi Regulation No. 4/2023 promotes mangrove-friendly silvofishery systems, including mangrove pens, yet implementation remains constrained by limited understanding of species-specific trophic niches of the two dominant cultured species, *Scylla olivacea* and *S. tranquebarica*. This study identified and quantified trophic niches of juvenile *S. olivacea* and *S. tranquebarica* and translated niche differentiation into ecosystem-based site-selection criteria. An integrated molecular–biogeochemical approach was applied in estuarine mangroves of Maros (South Sulawesi) and Segara Anakan Lagoon (Central Java), spanning salinity gradients of 5–26 ppt. DNA metabarcoding (Oxford Nanopore Technology) assessed ingested prey, while stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) evaluated assimilated carbon sources. Multivariate analyses, Standard Ellipse Area (SEA), and MixSIAR Bayesian models quantified dietary breadth and source contributions. Metabarcoding revealed no significant taxonomic prey differences (PERMANOVA, $p = 0.17$), indicating overlapping prey taxa. However, functional analyses demonstrated trophic segregation. *Scylla olivacea* primarily exploited heterotrophic, terrestrial–freshwater pathways, whereas *S. tranquebarica* relied more on autotrophic, mangrove-associated sources. Stable isotope results confirmed distinct basal carbon assimilation: S. olivacea derived nutrition mainly from detritus and biofilm (SEA = 3.11), while S. tranquebarica showed broader niche width (SEA = 9.29) and greater reliance on microphytobenthos and epiphyton. These findings link trophic niche differentiation to estuarine salinity gradients and habitat characteristics. Operationally, *S. olivacea* is best suited to brackish–freshwater, organic-rich inner mangrove zones, whereas *S. tranquebarica* is more suitable for brackish–marine creeks with active microphytobenthic production. The study provides evidence-based guidance for species-specific mangrove pen design, supporting ecosystem-based aquaculture and the implementation of provincial and national mangrove restoration policies.

Materials & Methods

Intro

Conventional grow-out pond systems have long supported the production of mud crabs (*Scylla* spp.), an important fisheries commodity in Indonesia. However, these systems have contributed to the degradation of nearly 600,000 ha of mangrove forests and are frequently associated with low survival rates and inconsistent growth, partly due to the inadvertent co-stocking of morphologically similar congeners. This practice conflicts with Indonesia's national commitment to halt mangrove deforestation and expand mangrove coverage under the Forestry and Other Land Uses (FOLU) Net Sink 2030 mission, which aims to enhance carbon sequestration while reducing greenhouse gas emissions.

At the provincial level, South Sulawesi Regulation No. 4/2023 (Article 20) mandates the development of mangrove-friendly silvofishery systems, including mangrove pens, as a sustainable alternative to conventional ponds. Mangrove pens rely on intact, multispecies mangrove ecosystems and their natural food webs to support crab growth. Despite this policy framework, implementation remains limited because the trophic niches of the two dominant cultured species, *Scylla olivacea* and *S. tranquebarica*, are poorly resolved. The absence of species-specific trophic and habitat criteria constrains evidence-based site selection and limits the operationalization of ecosystem-based aquaculture in mangrove landscapes.

This study aimed to (i) identify and quantify the trophic niches of juvenile *S. olivacea* and *S. tranquebarica* across estuarine mangrove systems; (ii) characterize functional dietary pathways and basal carbon sources supporting each species; and (iii) translate trophic niche differentiation into operational, ecosystem-based criteria for mangrove pen site selection, thereby supporting the implementation of provincial regulations and national FOLU Net Sink 2030 targets.

An integrated molecular–biogeochemical framework was employed to distinguish between ingested and assimilated dietary components. Ingestion pathways were assessed using DNA metabarcoding based on Oxford Nanopore Technology (ONT), while assimilation pathways were evaluated using stable isotope analysis (SIA). This complementary approach enables the separation of potential prey detection from the identification of food sources that are effectively incorporated into consumer biomass. Field sampling was conducted in estuarine mangrove systems in Maros (South Sulawesi) and Segara Anakan Lagoon (Central Java), encompassing contrasting salinity gradients and mangrove configurations. The BMRI by VLIZ 2025 grant facilitated the extension of metabarcoding to include plant-based dietary markers, thereby capturing the detrital and primary-producer components that underpin mangrove food webs. For DNA metabarcoding, fecal samples were collected from juvenile *S. olivacea* and *S. tranquebarica* and amplified using invertebrate and plant primers to resolve both heterotrophic and autotrophic dietary inputs. Prey composition was evaluated using multivariate analyses (PERMANOVA) and functional clustering based on prey feeding guilds and habitat categories.

For stable isotope analysis, 38 juvenile mud crabs, 29 intertidal crabs (as potential prey), and 11 fresh mangrove leaf samples were collected. Crab pereopod soft tissues and leaf samples were oven-dried at 60°C for 72 h, homogenized, and analyzed for $\delta^{13}C$ and $\delta^{15}N$ using an elemental analyzer coupled to an isotope ratio mass spectrometer. Dietary breadth and niche space were quantified using isospace analysis and Standard Ellipse Area (SEA), and source contributions were estimated using MixSIAR Bayesian mixing models.

Key environmental and ecological variables included estuarine salinity gradients (brackish–freshwater: 5–20 ppt; brackish–marine: 25–26 ppt), functional prey categories (heterotrophic versus autotrophic pathways; terrestrial–freshwater versus mangrove-associated sources), and basal carbon pools (detritus, biofilm, microphytobenthos, epiphyton, and sesarmid-derived material).

Results & Discussion

DNA metabarcoding revealed no significant differences in taxonomic prey composition among species, life stages, or locations (PERMANOVA, F = 1.46, R^2 = 0.39, p = 0.17), indicating high intraspecific variability. In contrast, functional clustering consistently differentiated the two species in Maros, demonstrating distinct trophic pathways. Juvenile *S. olivacea* primarily exploited heterotrophic, terrestrial–freshwater resources, whereas *S. tranquebarica* relied on autotrophic, mangrove-associated inputs. The prevalence of plant and minute invertebrate DNA indicates omnivorous and opportunistic feeding strategies that integrate macrophagous scavenging with microphagous detrital assimilation. These functional differences corresponded closely with species-specific salinity preferences, suggesting ecological segregation along estuarine gradients. Stable isotope analyses corroborated molecular findings by revealing greater dietary diversity in *S. tranquebarica* (SEA = 9.29) than in *S. olivacea* (SEA = 3.11). Although both species occupied comparable trophic positions, they differed in dominant basal carbon sources. *S. olivacea* exhibited stronger assimilation of sesarmid-derived and detrital carbon, whereas *S. tranquebarica* showed greater reliance on microphytobenthos and epiphyton. MixSIAR model outputs indicated that *S. olivacea* primarily derives nutrition from detritus and biofilm, while *S. tranquebarica* integrates a higher proportion of microphytobenthic and epiphytic sources.

The two species differed significantly in dietary niche width, with *S. tranquebarica* exhibiting an approximately threefold greater Standard Ellipse Area (SEA = 9.29) compared to *S. olivacea* (SEA = 3.11). No statistically significant segregation was detected at the taxonomic prey level (PERMANOVA, p = 0.17). In contrast, functional trophic segregation was evident, with consistent separation by feeding guild and habitat pathway, aligned with estuarine salinity ranges of 5–20 ppt for *S. olivacea* and 25–26 ppt for *S. tranquebarica*. Dominant basal carbon sources were identified as detritus and biofilm for *S. olivacea*, and microphytobenthos and epiphyton for *S. tranquebarica*.

This study provides integrated molecular and stable isotope evidence linking trophic niche differentiation in *Scylla* spp. to grow-out site suitability within mangrove ecosystems. The findings demonstrate that species-specific, functionally informed design of mangrove pens is critical for optimizing production outcomes while maintaining mangrove ecosystem integrity. Operationally, *S. olivacea* is best suited to brackish to freshwater transition zones characterized by soft, muddy, organic-rich substrates, such as inner mangrove basins, shaded creek margins, and litter-accumulating depressions. In contrast, *S. tranquebarica* is optimally cultured in brackish to marine mangrove creeks and sandy–mud flats exhibiting visible microphytobenthic films and epiphyte-covered prop roots or pneumatophores.

These results provide direct, evidence-based guidance for provincial stakeholders, including Dinas Perikanan dan Kelautan Provinsi Sulawesi Selatan, in implementing Regulation No. 4/2023. More broadly, the integration of functional molecular ecology into silvofishery planning advances a transferable framework for operationalizing the ecosystem approach to aquaculture in mangrove systems regionally and globally.

3. Overview of the expenditures

Describe in detail how the requested fund was spent within the implementation period (1 March 2025 and 28 February 2026). Be as specific as possible.

The BMRI grant was initially planned to support materials and devices for two analytical approaches applied in this study: Oxford Nanopore Technology (ONT) DNA metabarcoding and Stable Isotope Analysis (SIA). However, during the implementation phase, the actual expenditure required for the ONT metabarcoding approach exceeded the initially estimated budget. After several discussions with Professor Kochzius, it was therefore decided to allocate the EUR 5,000 BMRI grant exclusively to the ONT metabarcoding workflow, while the SIA approach was supported using alternative funding sources from the laboratory budget. The ONT metabarcoding pipeline consisted of three major stages: (1) two-step PCR amplification, (2) library preparation, and (3) sequencing. During implementation, additional methodological challenges emerged that had not been fully anticipated during the proposal stage. Although preliminary trials using Sanger

sequencing had been successfully conducted on crab tissue samples, working with crab faecal samples-particularly during PCR amplification-proved considerably more complex due to lower DNA quality and higher levels of inhibitors.
Multiple optimization trials were required before a reliable protocol was established. Three different PCR master mixes were tested (these optimization trials were not funded by the BMRI grant), and only one specific formulation proved suitable for amplifying DNA from faecal samples. Throughout this optimization process, several rounds of trial and error resulted in modifications to the original sequencing pipeline. Consequently, additional materials that were not initially planned became necessary to ensure successful sequencing. For example, SPRISelect Beads were required to perform pre-cleaning of PCR amplicons prior to library preparation, which significantly improved DNA quality and sequencing performance. These methodological adjustments ultimately enabled successful metabarcoding runs and the generation of robust and meaningful datasets.
The expenditures are detailed below (amounts in EUR):
ONT PCR: Phire Plant Direct PCR Master Mix= 263.78 (invoice: arida_invoice_1.pdf);
ONT PCR: Phire Plant Direct PCR Master Mix + Qubit dsDNA HS Assay Kit= 444.02 (invoice: arida_invoice_2.pdf);
ONT Library Preparation: Bovine Serum Albumin (BSA), Ultra Pure= 416.24 (invoice: arida_invoice_3.pdf);
ONT Library Preparation: SPRISelect Beads= 460.70 (arida_invoice_4.pdf);
ONT Sequencing Device: MinION Mk1D Standard (arida_invoice_5.pdf)= 2,871.00; and
ONT Sequencing Consumable: MinION Flow Cell R10 (arida_invoice_6.pdf)= 771.00.
Total expenditure: EUR 5,226.74
The slight excess beyond the original grant amount was covered by complementary laboratory funding. Overall, the BMRI grant played a critical role in enabling the successful completion of the ONT metabarcoding component of the project, which formed the core analytical foundation of the research outcomes achieved during the implementation period (March 1st 2025 - February 28th 2026). All invoices are attached separately from this report.

4. Pictures

A set of five pictures (low resolution in this document). The five high resolution pictures should be delivered to VLIZ by email to karen.rappe@vliz.be.

Figure 1. Collection of mud crabs in Sungai Ujung, Maros, South Sulawesi, Indonesia.

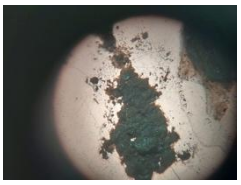

Figure 2. Faecal sample obtained from a mud crab Scylla olivacea.

Figure 3. Preparation and adjustment of PCR tubes prior to the two-step PCR protocol.

Figure 4. Quantification of PCR amplicons prior to downstream library preparation.

Figure 5. Adjustment of the MinION flow cell position prior to sequencing.