HAYATI
Journal of Biosciences

Vol. 33 No. 5, September 2026 1280-1290
DOI:10.4308/hjb.33.5.1280-1290
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Metabarcoding-Based Characterization of Diet Biota in the Gonggong Conch *Laevistrombus turturella* as a Potential Natural Feed Source

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ARTICLE INFO

Article history:
Received December 12, 2025
Received in revised form June 18, 2026
Accepted June 19, 2026
Available Online July 24, 2026

KEYWORDS:
Gonggong conch,
metabarcoding,
natural feed,
Ostracoda,
Skeletonema costatum

ABSTRACT

The gonggong conch *Laevistrombus turturella* is a highly valued seafood due to its excellent taste, high nutritional quality, and economic importance. However, increasing demand continues to rely on wild harvesting, raising concerns about overexploitation. Sustainable aquaculture is therefore essential, yet constraints persist, particularly regarding the suitability and availability of natural feed. Metabarcoding offers a high-resolution approach for identifying planktonic organisms as potential feed candidates, surpassing the accuracy of conventional visual methods. This study used DNA metabarcoding to characterize the gut content of *L. turturella* and to identify priority natural feed biota. The technique detected more than 10 times as many taxa as visual inspection, providing a clearer understanding of the species dietary profile. Ostracoda and Bacillariophyceae emerged as key candidate groups, with *Skeletonema costatum* and *Chaetoceros* spp. identified as the most suitable species based on their nutritional value, cell size, and culture tolerance. These findings provide a scientific basis for developing natural feed formulations to support the aquaculture of *L. turturella*.

1. Introduction

The gonggong conch *Laevistrombus turturella* is highly valued for its delicious taste and nutritional value (Muzahar *et al*. 2018a; Muzahar *et al*. 2019a; Viruly *et al*. 2024), as well as its substantial economic importance (Muzahar *et al*. 2021). This marine gastropod has long been consumed as a traditional side dish by communities in the Riau Islands Province (Muzahar & Hakim 2018b; Viruly *et al*. 2019), particularly in Tanjungpinang City. Demand for gonggong conchs has continued to increase in line with population growth over the last two decades; the population of Tanjungpinang City reached 237,580 inhabitants in 2024 (BPS Tanjungpinang 2024). Currently, all supplies rely entirely on wild harvesting. However, recent observations indicate declining catch volumes and reduced individual sizes, suggesting ongoing overexploitation. This situation poses a significant threat to the species' sustainability and continued use.

Aquaculture development of the gonggong conch has the potential to reduce harvesting pressure on wild populations (Muzahar *et al*. 2019b). Techniques for distinguishing male and female individuals have already

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been established, with broodstock sizes identified as ≥6.3 cm for males and ≥6.7 cm for females (Muzahar *et al*. 2020a). Initial hatchery trials have been conducted; however, progress remains constrained by the availability of appropriate natural feed during the larval stage, when *L. turturella* requires exogenous feeding. Muzahar *et al*. (2020b) provided the phytoplankton *Nannochloropsis* sp. as a natural feed for gonggong conch larvae, but survival rates remained low. The species is known to be a selective feeder (Nezaputri *et al*. 2021), underscoring the importance of identifying suitable natural food organisms to support successful larval rearing. Therefore, identifying natural feed types that match the mouth opening size of gonggong conch larvae and juveniles is essential for successful hatchery production. One approach to identifying potential biota suitable as natural feed is to study dietary patterns. Information on the diet of *Laevistrombus turturella* remains limited, particularly regarding variations associated with habitat type, body size, and sex. DNA metabarcoding of gut contents offers a powerful tool for identifying the diverse biota consumed by this species. To date, no studies have applied metabarcoding techniques to examine the gut contents of gonggong conchs from the littoral zone of Madong Bay, Tanjungpinang City. Accordingly, this research is crucial for identifying appropriate natural feed sources to support the aquaculture of this species. The objective of this study was to analyze the natural dietary biota consumed by *L. turturella* using gut-content metabarcoding (Pratomo *et al*. 2022). The biota species identified as dominant or consistently consumed will be proposed as candidate natural feed organisms for gonggong conch aquaculture.

2. Materials and Methods

2.1. Study Period and Location

The study was conducted in the littoral zone of Madong Bay, Tanjungpinang City, Riau Islands Province, Indonesia. Sampling was carried out from August to December 2025. Gonggong conch samples were collected at coordinates 0.9827-0.9835°N and 104.43425-104.4637°E in the littoral zone of Madong Bay, Tanjungpinang City, Indonesia (Figure 1). Gut content analysis using metabarcoding was performed at the Oceanogen Laboratory in Bogor, Indonesia. At the same time, a visual examination was conducted at the Marine Biology Laboratory of Raja Ali Haji Maritime University in Tanjungpinang City.

2.2. Research Procedure

2.2.1. Gonggong Conch Sample Collection and Handling

Gonggong conchs were manually collected by hand from their natural habitat in the littoral zone of Madong Bay, Tanjungpinang. All specimens were thoroughly rinsed, then subjected to morphometric measurements and determination of total body weight. The shells of the conchs were carefully cracked using a hammer to extract the soft tissue and to determine the sex of each individual. The sex ratio of female to male gonggong conchs was calculated using the following formula:

$$\text{Sex ratio of gonggong conchs} = \text{Number of males: Number of females}$$

The gut contents of the gonggong conchs were first measured for total length and then weighed to determine total weight. The intestines from two individuals were separated from the gut contents, placed into 1.5 mL tubes containing DNA Shield (a DNA preservation solution), and stored at -20°C for subsequent metabarcoding analysis. In addition, a visual analysis of the gut contents was conducted as follows: the intestines of five gonggong conchs were separated from the body and carefully dissected with surgical instruments. The gut contents were scraped out, minced, and diluted with 1 mL of distilled water. The diluted contents were then examined under a microscope to identify and observe their composition.

2.2.2. Gonggong Conch Gut Content Analysis Using Metabarcoding

The analysis consisted of DNA extraction, PCR amplification, and sequencing to obtain DNA from organisms that constitute the diet of gonggong conchs. The procedures were carried out as follows:

(1) Gut content collection and extraction

A total of two gut contents of *Laevistrombus turturella* were collected from two different individuals. Gut content samples were then preserved in prefilled 2 mL cryotubes with the DNA shield (ZymoBIOMICS DNA/RNA shield). Decontamination was performed by strictly sterilizing all sampling equipment at each stage of the sampling procedure using a 10% commercial bleach solution. The samples were then transported to Oceanogen Lab in Bogor, Indonesia, for further laboratory analysis. The eDNA samples and blank

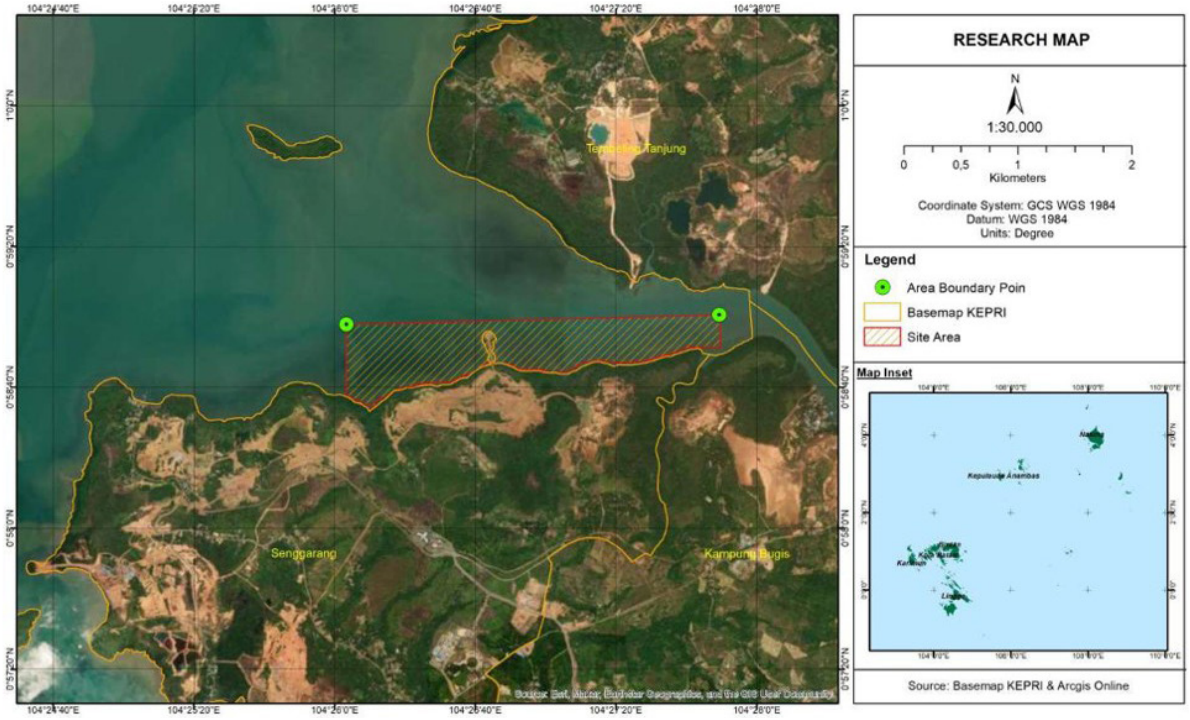

Figure 1. Study site in the littoral zone of Madong Bay, Tanjungpinang City, Indonesia

DNA template were then processed using the Quick-DNA Microprep Plus Kit (Zymo Research) following the manufacturer's protocol. For the final DNA extraction products, we performed two 50 L elutions, for a total extract volume of 100 L, to obtain more DNA material.

(2) Library preparation and next-generation sequencing

DNA amplification was performed using V9 hypervariable regions of the eukaryotic small subunit (SSU) 18S ribosomal RNA (rRNA) genes and prepared for 2×250 bp paired-end Illumina MiSeq sequencing (Illumina, San Diego, CA, United States) using V9 primer set 1389F: 5′-TTG TAC ACA CCG CCC-3′ and 1510R: 5′-CCT TCY GCA GGT TCA CCT AC-3′ according to Stoeck *et al*. (2010) with high-fidelity PCR hot start to gain more accurate amplification of DNA or PCR products. The first PCR reaction contained a 12 µL reaction mixture consisting of 6 µL of Kapa HotStart HiFi 2× ReadyMix DNA polymerase (Kapa Biosystems Ltd., London, UK), 2 µL of ddH_2O, 0.8 µL each of the forward and reverse primers, and 2.4 µL of DNA template. All primer sequences were inserted with a unique adaptor sequence, following the Nextera UD Index protocol, for further indexing PCR. The thermocycle procedure used the following PCR conditions: pre-denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 98°C for 20 seconds, annealing at 67°C for 15 seconds, and extension at 72°C for 15 seconds; and a final extension at 72°C for 10 minutes.

(3) Bioinformatics Analysis

The DNA sequence data in FASTQ format were first quality-checked using FastQC v.0.11.8. Sequences shorter than 80 base pairs (bp) or with a quality Phred Q score below 20 were removed using Cutadapt v.1.18 (Martin 2011). Subsequently, Qiime2.2019.10 pipeline (Caporaso *et al*. 2010; Bolyen *et al*. 2019) was employed for using DADA2 v.2018.11.0 (Callahan *et al*. 2016) (via q2-dada2), which was applied for denoising, joining denoised paired-end reads, filtering out chimeric sequences and singletons, and dereplicating sequences to produce amplicon sequence variants (ASVs) as proxies for species. ASVs were taxonomically classified using BlastN with a percentage identity ≥98% against the Eukaryota reference database, Primer 18S CRUX (https://ucedna.com/reference-databases-for-metabarcoding), which generates comprehensive reference databases for specific user-defined metabarcoding loci. (Curd *et al*. 2019). Based on WORMS (https://www.marinespecies.org/), the obtained eukaryotic taxonomy was further analyzed to determine taxonomic ranks from kingdom to species. DNA decontamination analysis followed the procedure of Port *et al*. (2016), where the BLAST identity percentage was > 98% against the 18S rRNA

gene database, removal of low-frequency ASV data (< 0.0001%), and 'exotic' taxa found, namely ASVs detected from fossil and terrestrial biota. These 'exotic' taxa were assumed not to be components of gonggong's diet (see Supplement 2). Therefore, these ASVs were excluded from further analysis. To resolve taxonomic ambiguities within the "gonggong" group, a phylogenetic analysis was conducted using reference Strombidae sequences from the NCBI database, including a representative *Laevistrombus* sp. ASVs were aligned with these references using MAFFT (Katoh & Standley 2013) via the EMBL-EBI JDispatcher platform (https://www.ebi.ac.uk/jdispatcher/). Subsequently, a neighbor-joining tree was constructed using default parameters (distance correction, exclude gaps, and percent identity matrix were off) and visualized via iToL (https://itol.embl.de/) to clarify the evolutionary relationships of the taxa.

2.3. Data Analysis

The obtained data were analyzed descriptively and presented in figures and tables, with discussions contextualized and supported by relevant scientific literature. Taxonomic composition was visualized using a Krona diagram (https://github.com/marbl/Krona/wiki) and Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/). Diet composition and field-sampled biodiversity of the gonggong conch were estimated following Magurran (2005). Biodiversity was quantified via the Shannon-Wiener Index (H'), representing both richness and evenness, and Pielou's Evenness Index (E'), which scales from 0 to 1 to measure the numerical equitability of species populations. While H' provides a measure of overall community complexity, the Dominance Index (D') was specifically applied to assess the relative concentration of the most abundant taxa.

3. Results

3.1. Morphometrics, Meristics, and Sex Composition of Gonggong Conchs

A total of 97 gonggong conchs were successfully collected from the study site, and their morphometric and meristic data, along with the number of individuals by sex, are presented in Table 1.

3.2. eDNA Sequence Data Acquisition

Gut contents samples were collected from the littoral zone of Madong Bay, Tanjungpinang City. DNA extraction, PCR amplification, and sequencing using metabarcoding techniques were subsequently performed to identify the DNA of organisms representing the presumed diet of the gonggong conchs. Visual examination of the gut contents from the same samples was also conducted for comparison. Most of the gut organisms were already degraded, making identification difficult. Nevertheless, four genera could be identified from relatively intact organisms, *namely Nitzschia sp., Chlorella sp., Navicula sp.,* and *Prorocentrum* sp. (Figures 2B-E).

3.3. DNA Acquisition from the Gut Contents of *Laevistrombus turturella*

Visualization of PCR results from gut content samples of *Laevistrombus turturella* using 2% agarose gel electrophoresis showed a band at approximately 250 bp (Figure 3). Both samples were successfully sequenced, yielding a total of 295,284 DNA sequences (Table 2). After processing with the DADA2 algorithm, 163,073 sequences were retained, yielding 285 amplicon sequence variants (ASVs) designated ASV001-ASV285. Amplicon Sequence Variants (ASVs) represent representative DNA sequences corresponding to potential individual organisms of a specific type. The results also revealed 54 ASV sequences shared between the two samples, representing the common organisms consumed by the gonggong conchs in these samples.

3.4. Identification of Amplicon Sequence Variants (ASVs)

ASV sequences were identified using BlastN against the Eukaryota reference database Primer 18S CRUX (https://ucedna.com/reference-databases-for-metabarcoding). The results showed that over 99% of the sequences originated from *L. turturella* DNA,

Table 1. Morphometric and meristic data and number of individuals by Sex of Gonggong Conchs

Sex	Average shell length (cm)	Average total weight (g)	Average flesh weight (g)	Average visceral length (cm)	Average gonad length (cm)	Average gonad weight (g)
Male: 55	5.703±0.449	20.977±4.577	6.307±1.181	9.960±2.522	2.929±0.682	0.701±0.216
Female: 42	5.964±0.474	24.461±6.152	8.404±1.986	10.009±2.415	3.269±1.091	1.088±0.519

The sex ratio of gonggong conch (male: female) was 1.00:0.76

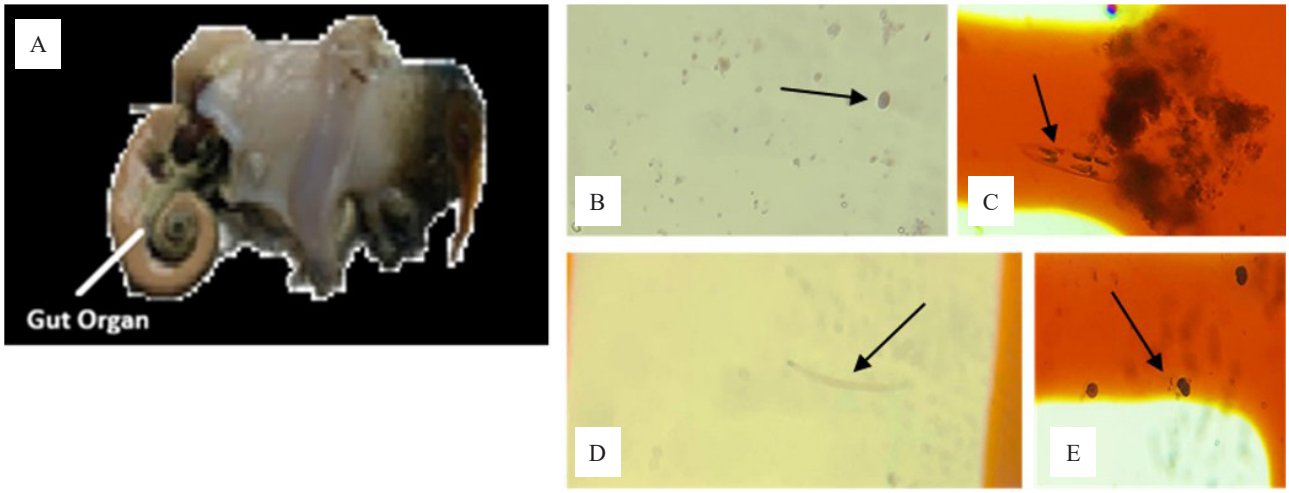

Figure 2. (A) Gonggong conch intestine and food particles in the gut contents were observed visually. (B-E) *Chlorella* sp., *Navicula* sp., *Nitzschia* sp., and *Prorocentrum* sp.

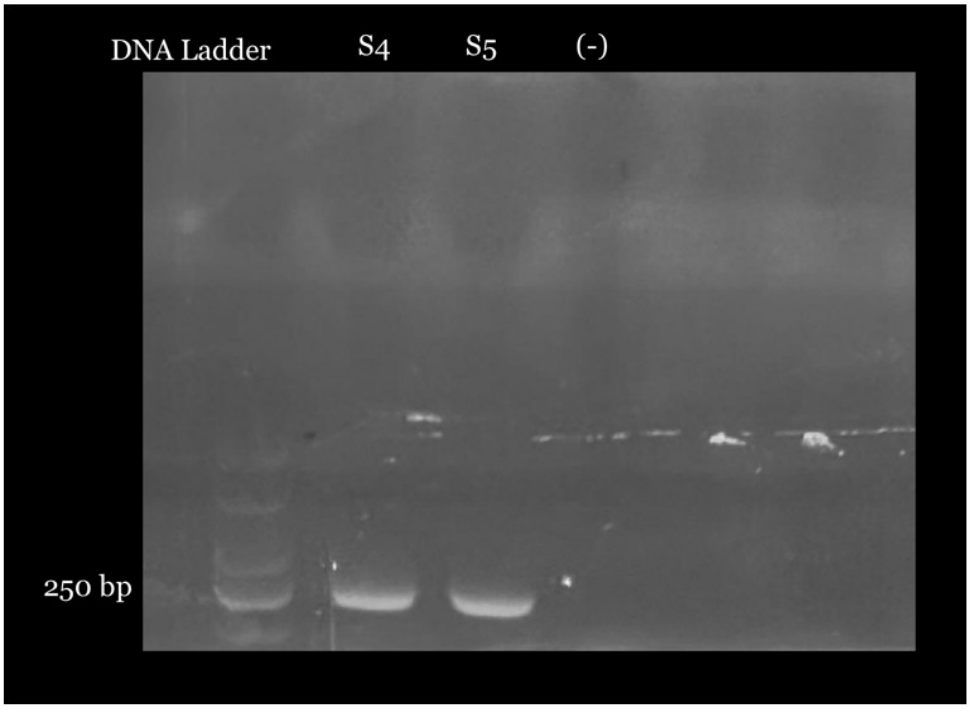

Figure 3. Electrophoresis gel result for the amplicon products

Table 2. DNA sequence yield from the gut contents of *Laevistrombus turturella*

Description	Sample 01	Sample 02	Total
Total Sequences	149.949	145.335	295.284
Sequences after DADA2	80.115	82.956	163.071
Total ASVs	176	163	285
Clean Sequences*	25.182	12.051	37.233
Clean ASVs*	156	118	228

*After decontamination

indicating that less than 1% derived from other organisms present in the gut contents of the gonggong conchs (Supplement 1).

4. Discussion

Table 1 shows that all morphometric and meristic values of female *Laevistrombus turturella* were larger than those of males, except for the number of individuals

observed. This pattern is consistent with findings reported by Cob *et al*. (2009) for *Strombus canarium* and by Avila-Poveda & Cardenas (2006) for *Strombus gigas*. However, fewer female gonggong conchs were recorded during the present study, resulting in a male-to-female ratio of 1.00:0.76. This ratio differs from that reported by Brillantes *et al*. (2024) for *Laevistrombus (Strombus) canarium* in Samar, Philippines (male: female = 1.00:1.99), Widyastuti & Aji (2016) for *Lambis lambis* along the Yenusi coast, Biak (1.00:1.20), and Wawo *et al*. (2024) for four *Canarium* species on Saparua Island, Maluku, Indonesia (1.00:1.00). Variations in male-to-female ratios among marine gastropods, including Strombidae, are influenced by multiple factors, such as seasonal changes, inter site variability, individual size, sampling period, and the sex-determination techniques employed. Differences in the intestinal length of the gonggong conch may influence the amount of food biomass it can accommodate. The longer intestine of the female gonggong conch, compared to that of the male, provides a greater capacity to consume more abundant and diverse food items. Consequently, the probability of obtaining total dietary DNA sequences from the female individual is higher than from the male individual. The total number of DNA sequences in the female individual was greater than in the male individual, as shown in Table 2. Therefore, this condition may enhance the detection of more taxa using the metabarcoding approach.

The Amplicon Sequence Variant (ASV) results, in which over 99% of sequences originated from *Laevistrombus turturella*, were expected because the primary DNA source came from residual gut tissue of the sampled conchs. This dominance can hinder the amplification of low-abundance DNA during PCR, suggesting that many ASVs may remain undetected (Vestheim & Jarman 2008). Such limitations could be mitigated by applying a "Primer Blocking" procedure to optimize the detection of gut organisms (Ascher *et al*. 2025); however, this approach was not feasible in the present study due to high costs. During decontamination following the method of Port *et al*. (2016), *L. turturella* DNA was removed along with all DNA derived from terrestrial and fossil organisms. The results are presented in Supplement 2. The remaining 228 ASV sequences were retained to represent gut organisms for further analysis of the dietary composition of the gonggong conchs (Table 2; Supplement 3).

The feeding behavior of adult gonggong conchs is omnivorous, with detritus ingested via the proboscis (Cob *et al*. 2014; Nezaputri *et al*. 2021). Using this mechanism, provided that particle size is compatible with the feeding organ, both dead and living organisms may be consumed. Visual examination of the gut contents confirmed the ingestion of intact living biota, specifically algae (Figures 2B-E).

The metabarcoding analysis proved highly effective, with only 9.32% of the total ASVs remaining taxonomically unidentified. According to the World Register of Marine Species (WoRMS), all identified taxa were marine, with very few from brackish or freshwater environments. The gut contents were predominantly derived from marine biota, with a minor proportion representing highly diverse organismal groups: five kingdoms, 27 phyla, 48 classes, 78 orders, 90 families, and 74 genera were detected. The kingdoms were dominated by Animalia (58.80%) and Chromista (39.75%), while the remaining 1.45% belonged to Plantae, Fungi, and Protozoa (Figure 2A). In total, 74 genera were identified. These findings indicate that gonggong conchs are multitrophic, yet exhibit strong dietary preferences. The subsequent discussion focuses on the two dominant groups.

Within the gut contents of the gonggong conchs, the kingdom Animalia comprised seven phyla, eight classes, 11 orders, 11 families, and eight genera, with five ASV-level genera remaining unidentified. Animalia was overwhelmingly dominated by Arthropoda, accounting for 99.27% and consisting primarily of the genus *Loxocorniculum*, a member of the class Ostracoda (Figure 2A). This genus was detected from ASV005 to ASV010. *Loxocorniculum* was first reported from East Asian waters, including Japan and Korea (Yoo & Karanovic 2019), but has not previously been reported in Indonesia. These organisms likely belong to the genus *Loxoconcha*, which has been recorded in Jakarta Bay (Fauzielly 2013). However, to confirm the taxonomic identity, further investigation of benthic biota in the gonggong conch habitat is required. Nevertheless, Ostracoda are biogeographically widespread, including in Indonesia (Titterton & Whatley 1988). Ostracods are small benthic crustaceans that primarily inhabit sediments; 94 species have been reported in Jakarta Bay, Indonesia (Fauzielly 2013). These findings suggest that benthic organisms, such as Ostracoda, are a primary dietary preference for gonggong conchs in the littoral zone of Madong, Tanjungpinang City. To the best of our knowledge, no previous studies on the diet of gonggong conchs in Indonesia have reported this taxon. Similar observations have been made in other studies, where Ostracoda were detected in *Strombus canarium* diets alongside Hydrozoa and Gastropoda (Cob *et al*. 2014).

The gut contents of gonggong conchs from the Chromista group were more diverse than those of Animalia, comprising 13 phyla, 26 classes, 51 orders, 63 families, and 57 genera, with 41 ASVs remaining unidentified. The composition was dominated by three major phyla: Heterokontophyta (diatoms) (68.14%), Cryptophyta (13.33%), and Myzozoa (6.86%). Within the Animalia genera, nine main genera were identified, with Nitzschia representing the largest proportion (14.43%), followed by *Navicula*, *Thalassiosira*, *Seminavis*, *Campylodiscus*, *Prorocentrum*, and *Skeletonema*, collectively accounting for 53.9% of this group. The remaining 50 genera each comprised less than 3% of ASVs, with 22.28% of ASVs unassigned at the genus level (Figure 3C). Previous studies also reported that Chromista are dominant in the gut contents of gonggong conchs (Cob *et al*. 2014; Yunita *et al*. 2021; Nezaputri *et al*. 2021), including Bacillariaceae genera such as *Navicula*, *Nitzschia*, *Synedra*, and *Thalassiosira* (Nezaputri *et al*. 2021).

A survey of benthic and planktonic biota in the surrounding waters of the Gonggong conch sampling site was conducted as a comparison. The survey identified only Chromista and Animalia, comprising 6 phyla, 8 classes, 31 orders, 49 families, and 56 genera (Figures 4B and 5A). When the taxa detected in the gut contents were compared with those present in the littoral zone of Madung, eight genera were found in both datasets: *Amphora*, *Biddulphia*, *Campylodiscus*, *Chaetoceros*, *Cyclotella*, *Navicula*, *Nitzschia*, and *Surirella* (Figure 5B). All of these genera belong to the class Bacillariophyceae, and only *Navicula* and *Nitzschia* were successfully identified visually in the gut contents. The difference in biotic composition between the metabarcoding-based gut content data and the field survey of the littoral zone was substantial, with a Jaccard similarity index of only 6.45% (100% indicates no difference). This distinct disparity reflects the high sensitivity of the metabarcoding approach in detecting organisms at the molecular level. Comparative analysis of biodiversity metrics revealed a higher degree of taxonomic complexity in gut content samples than in the littoral zone, as evidenced by H' values of 3.14 and 2.58. These elevated values suggest that DNA metabarcoding provides a more exhaustive characterization of community stability and species richness than traditional field surveys. While E' values were marginally higher in the field survey (0.58 and 0.63), the D' values remained comparably low across both datasets (0.11 and 0.14), respectively. Collectively, these findings underscore the superior sensitivity of molecular approaches in detecting cryptic or overlooked taxa within the ecological assemblage.

The gonggong conch belongs to the family Strombidae and is a benthic organism with detritivorous feeding habits and an omnivorous diet. It uses its radular apparatus to scrape or graze algal or epiphytic layers, which are then ingested through the proboscis (Cob *et al*. 2014; Nezaputri *et al*. 2021). Geographically, this species is widely distributed worldwide. Most previous studies have reported that the gut contents of *Laevistrombus* spp. are predominantly composed of Bacillariophyceae. However, the present study reveals a previously unreported finding: Ostracoda constitutes the primary dietary component of the gonggong conch. Ostracods are benthic microcrustaceans measuring 0.1-1 mm, possessing a hard carapace composed of chitin and calcareous material. Experimental culture trials have demonstrated that ostracod densities can increase from 20-30 individuals per liter to as many as 2,050 individuals per liter, indicating their potential as a natural feed source for the gonggong conch (Chhaba 2021).

Among the Ostracoda species that have been successfully cultured and utilized in marine aquaculture, *Heterocypris salina* (Cypridoidea) is particularly notable for its rapid reproductive capacity, ability to grow efficiently on microalgae, and suitable size for the larvae of several fish species, as reported by Yousef & Hegab (2017). Several Bacillariophyceae phytoplankton taxa identified in this study also show strong potential as natural feed candidates for Laevistrombus hatchery programs, as they have already been successfully mass-cultured in Indonesia. These include *Skeletonema costatum*, *Chaetoceros* spp., *Navicula* spp., *Nitzschia* sp., and *Thalassiosira pseudonana*. Among these, two species-(1) *Skeletonema costatum* and (2) *Chaetoceros* spp.-are widely used as natural feed for shrimp and fish larvae in commercial hatcheries.

The gonggong conch has strong aquaculture potential, as it is widely consumed and holds significant economic value, particularly in Indonesia, Malaysia, Vietnam, Thailand, the Philippines, and India (Cob *et al*. 2014). However, its taxonomic description remains problematic, with frequent synonymy and confusion among the genera Strombidae, Canarium, Laevistrombus, and Dolomena (Maxwell *et al*. 2019; Dekkers & Maxwell 2020). According to WoRMS (accessed December 2025), Laevistrombus turturella has been reassigned to the genus *Dolomena*, now recognized as *Dolomena turturella*. Phylogenetic analysis of the conch DNA sequences generated in this study (ASV160–165, previously

identified as *L. turturella*) compared with reference SSU18S Strombidae sequences from NCBI indicates a polyphyletic pattern (Figure 6). This finding further confirms the ongoing taxonomic ambiguity surrounding this species complex. A comprehensive redescription—particularly within the context of aquaculture standards—is urgently needed to determine whether the currently consumed gonggong conch represents a single species,

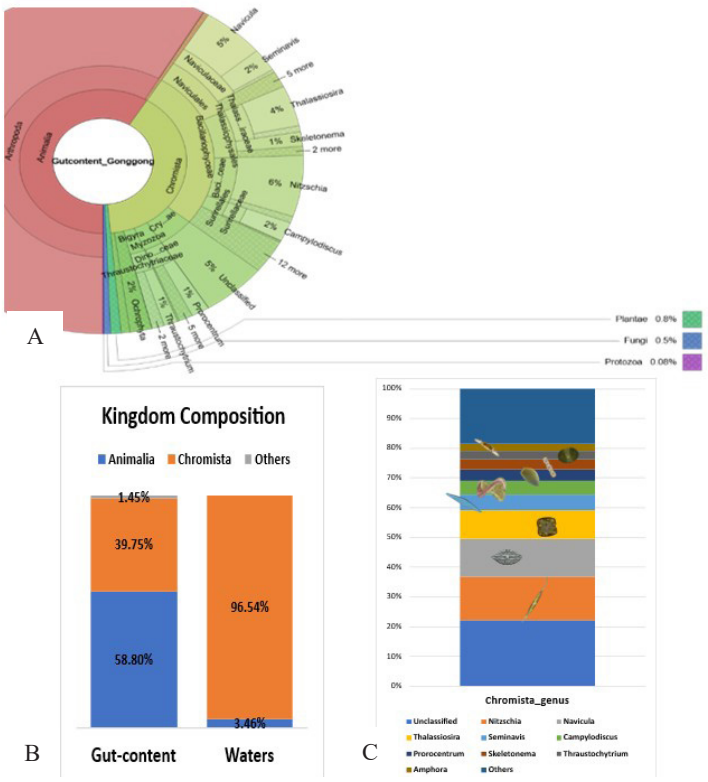

Figure 4. (A) Krona diagram showing the composition of biota in the gut contents. (B) Comparison of kingdom-level composition between gonggong conch gut contents and water column biota in Madong Bay. (C) Gut content composition of gonggong conchs at the genus level within the Chromista group

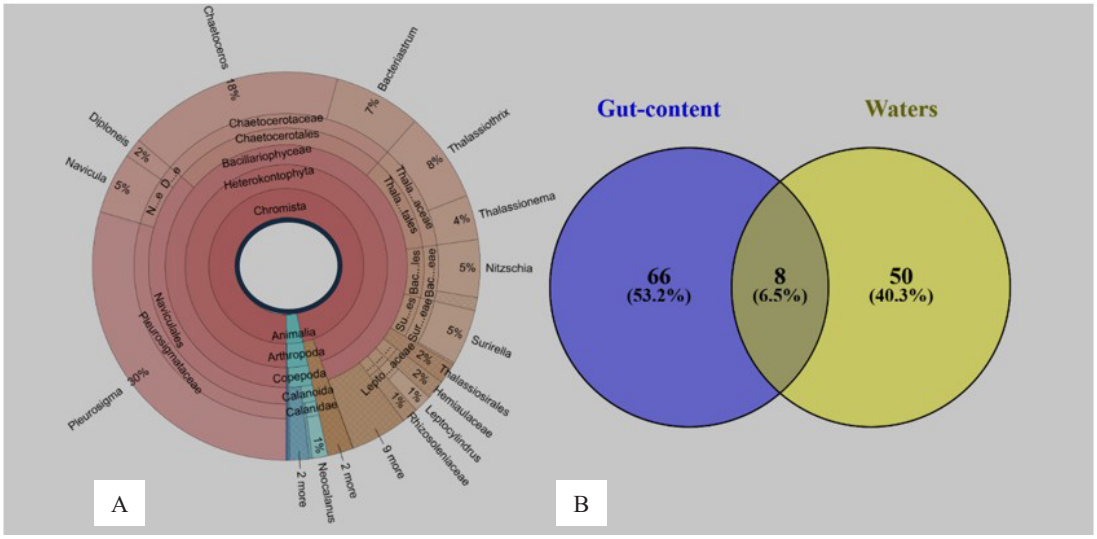

Figure 5. (A) Krona chart of the biotic composition in the waters of Madung Bay. (B) A Venn diagram showing the overlap between the gut contents of the gonggong conch and aquatic biota in Madung Bay at the genus level

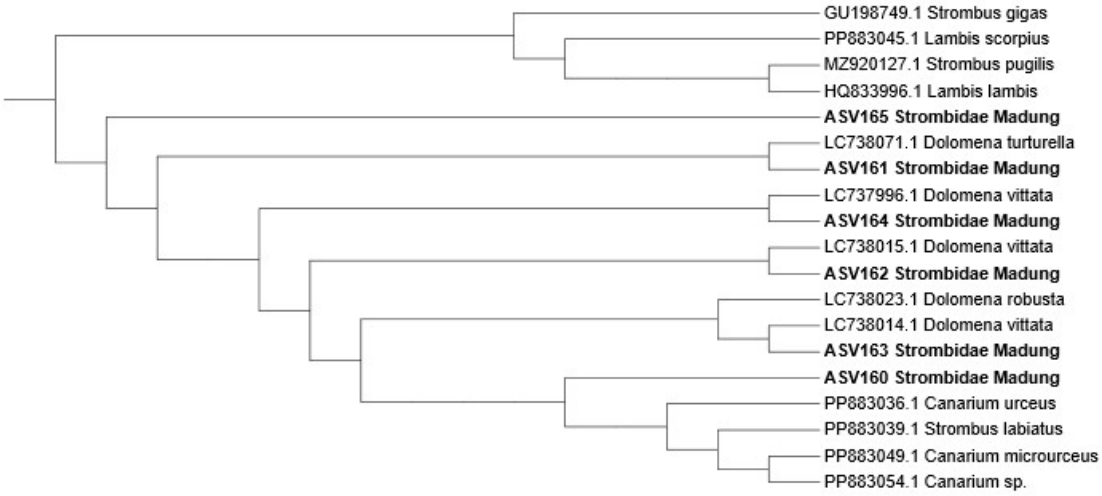

Figure 6. Phylogenetic relationships of the gonggong conch based on gut-content DNA obtained in this study, shown in comparison with selected members of the family Strombidae

multiple distinct species, or merely morphological variants of one another.

In Conclusion, The findings of this study demonstrate that gut-content DNA metabarcoding provides a substantial advantage, detecting more than 10 times as many organisms as conventional visual examination. These results offer important insights into the dietary patterns of the gonggong conch. They also indicate that priority natural feed candidates for potential development include organisms from the Ostracoda and Bacillariophyceae groups.

Acknowledgements

This research was funded by Raja Ali Haji Maritime University. The authors express their sincere gratitude to the Rector of Raja Ali Haji Maritime University, the Head of the Institute for Research and Community Service, and the Head of the Marine Biology Laboratory, Faculty of Marine Science and Fisheries, for providing facilities and support. We also extend our appreciation to the students of the Aquaculture, Aquatic Resource Management, and Fishery Product Technology Study Programs at Raja Ali Haji Maritime University, namely Mega, Aliyah, Riri, Dicky Albert, Tri Nurdinda, Azri, Syakban, M. Raihan, and Chandra, for their contributions to this study, as well as to all individuals who assisted the authors in any capacity.

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