

Microbial Abundance and Coliform Contamination in the Gut of *Lambis lambis* from Recreational Water of Cowrie Island, Honda Bay, Palawan

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ABSTRACT

The gut microbiota of marine mollusks reflects both their dietary inputs and exposure to environmental contaminants, making them valuable indicators of microbial ecology in coastal ecosystems. This study investigated the microbial abundance and coliform contamination in the gut of *Lambis lambis* collected from Cowrie Island, Honda Bay, with the objectives of quantifying the abundance of microbial bacteria and fungi and determining coliform levels using the Most Probable Number (MPN) method. A total of ten (10) adult specimens were collected and analyzed using culture-based microbiological techniques. Microbial abundance was determined through serial dilution and plate count methods. Coliform levels were assessed using the conventional three-tube MPN method. Results showed that bacterial populations were consistently higher than fungal populations, with bacterial counts ranging from 19.5×10^4 to 57.1×10^4 CFU/g, while fungal counts ranged from 1.67×10^4 to 7.7×10^4 CFU/g. Coliform analysis revealed MPN values ranging from 150 to 460 MPN/100 mL, all exceeding the recommended standard limit of 100 MPN/100 mL. The elevated coliform counts indicate exposure of *L. lambis* to contaminated marine environments, possibly influenced by anthropogenic activities such as tourism and coastal pollution. The findings suggest that bacteria dominate the gut microbiota of *L. lambis* and that the species may serve as a potential bioindicator of microbial pollution in coastal ecosystems. This study provides baseline information on the microbial ecology and environmental conditions of recreational marine waters in Honda Bay, Palawan.

Keywords: microbial abundance, coliform contamination, Most Probable Number, gut microbiota, coastal pollution.

INTRODUCTION

The Common Spider Conch, *Lambis lambis* (Linnaeus, 1758), locally known as “Saang” or “Rangara” in the Philippines, is a marine gastropod belonging to the family *Strombidae*. This species is widely distributed throughout the Indo–West Pacific region and commonly inhabits shallow coastal ecosystems such as coral reefs, seagrass beds, reef flats, and mangrove-associated habitats (Abbott 1960; Poutiers 1998). In the Philippines, particularly in Palawan, *L. lambis* is considered an ecologically and economically important marine resource that contributes to artisanal fisheries and local food security (Bellchambers et al. 2011; Rabia et al. 2023).

Gut-associated microorganisms are essential components of marine invertebrate physiology because they contribute to digestion, nutrient cycling, metabolism, and host health (Nayak 2010; Wei et al. 2018). The gut microbiota of marine gastropods is generally composed of diverse bacterial and fungal communities influenced by environmental conditions, diet, and habitat quality. However, despite the ecological importance of *L. lambis*, limited information is available regarding its gut microbial abundance and associated microbial contamination in coastal marine ecosystems, specifically in recreational waters.

Recreational marine waters are vulnerable to microbial pollution resulting from anthropogenic activities such as tourism, sewage discharge, coastal development, and improper waste disposal (Akita et al. 2021). Coliform bacteria are widely used as indicators of fecal contamination and environmental sanitation (Rompré et al. 2002). Elevated coliform levels in marine organisms may indicate exposure to polluted waters and possible public health risks associated with seafood consumption. In tourist destinations such as Cowrie Island in Honda Bay, increasing human activities may contribute to microbial contamination in coastal habitats (Cayabo et al. 2021).

Given the dual importance of *L. lambis* as both a food resource and a sentinel organism in coastal ecosystems, this study aimed to (1) characterize the bacterial and fungal abundance in the gut microbiota of *L. lambis*, and (2) assess coliform contamination levels of this species collected from Cowrie Island, Honda Bay, Palawan. The results will provide baseline microbiological data to evaluate the sanitary quality of recreational waters and the potential use of *L. lambis* as a bioindicator of coastal microbial pollution in the region.

METHODS

Study Site and Sample Collection

The sample collection was conducted on December 10, 2025 at the recreational marine waters of Cowrie Island (9°50'23"N 118°46'05"E) located in Honda Bay (Figure 1).

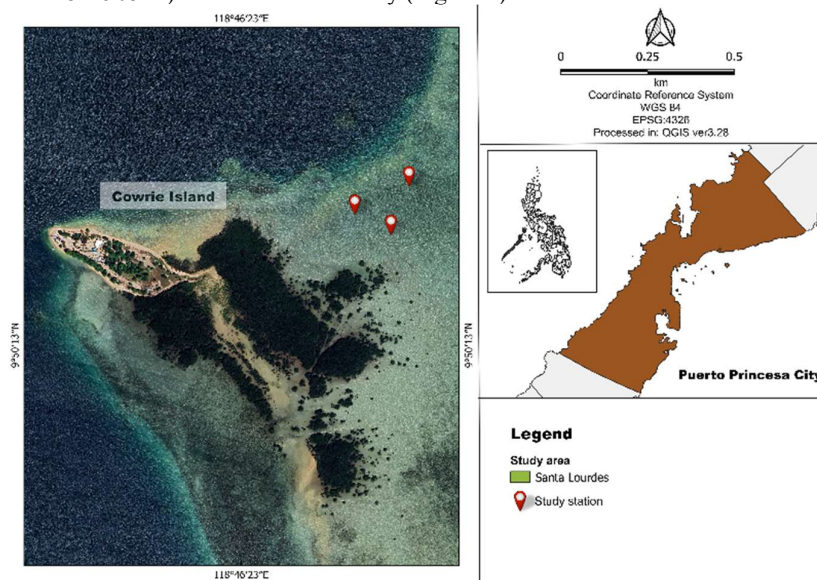


Figure 1. Map showing the locale of the study at Cowrie Island, Honda Bay, Puerto Princesa City, Palawan.

A total of ten (10) adult specimens of *Lambis lambis* were collected through handpicking and shallow diving. Collected specimens were individually placed in sterile plastic containers containing ambient seawater and transported to the laboratory using an ice cooler. Species identification was based on shell morphological characteristics following the taxonomic guide of Poutiers (1998) (Figure 2). Prior to dissection, the shell surfaces were cleaned and sterilized using 70% ethanol followed by sterile distilled water rinsing to minimize external contamination.



Figure 2. Picture of Genus *Lambis* cited in the book of “The living marine resources of the Western Central Pacific Volume 1” (Poutiers, 1998).

Dissection and Sample

Using aseptic techniques, the digestive tract of each specimen was carefully isolated. Gut contents were aseptically removed and homogenized using a sterile mortar and pestle. The homogenized samples were combined into three composite samples for microbial analyses. Approximately 10 g of homogenized gut sample was mixed with 90 mL of sterile distilled water to prepare the stock suspension for serial dilution and microbiological examination.

Quantification of Bacterial and Fungal Abundance

The microbial abundance was quantified using the serial dilution and plate count method following Mabuhay et al. (2004). Serial dilutions up to 10^{-4} were prepared from the homogenized gut suspension. For total bacterial count, Nutrient Agar (NA) was used. This medium was suitable for enumerating heterotrophic bacteria from marine samples. Bacterial plates were incubated at 35 °C for 24 hours (Mabuhay et al. 2004). For fungal enumeration, Potato Dextrose Agar (PDA) was used and incubated at 35°C for seven (7) days. After incubation, visible colonies were counted and expressed as colony-forming units per gram (CFU/g) using the formula:

$$CFU/g = (\text{average number of colonies} \times \text{reciprocal of dilution factor}) / \text{volume plated (mL)}$$

Most Probable Number (MPN) of Coliform

Coliform levels were determined using the conventional three-tube Most Probable Number (MPN) method following Rompré (2002). Homogenized gut samples were inoculated into lactose broth tubes containing Durham tubes. Double-strength lactose broth tubes received 10 mL inoculum, while single-strength lactose broth tubes received 1 mL and 0.1 mL inocula, respectively. All inoculated tubes were incubated at 35°C for 24–48 hours. Tubes exhibiting gas production and turbidity were considered positive for coliform bacteria. The number of positive tubes was compared with the standard MPN reference table to estimate coliform density expressed as MPN/100 mL (Table 1).

Data Analysis

Descriptive statistics such as mean and standard deviation were used to summarize bacterial and fungal abundance expressed as CFU/g and coliform levels expressed as MPN/100 mL. Coliform results were interpreted based on the standard MPN reference table following Rompré (2002) (Table 1).

Table 1. Most Probable Number (MPN) of Coliform Reference Table.

MPN Determination for multiple tube tests numbers of tubes giving positive reaction out of			MPN index per 100 mL	94% confidence limit	
3 of 10mL each	3 of 1mL each	3 of 0.1mL each		Lower	Upper
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1,300
3	3	1	460	71	2,400
3	3	2	1,100	150	4,800
3	3	3	>1,400		

From: Standard Method for Examination of water and wastewater, twelfth edition. (New York: The American Public Health association, Inc., p. 608)

RESULTS

Microbial Abundance (Bacteria and Fungi)

The bacterial populations in the gut of *L. lambis* ranged from 19.5×10^4 CFU/g to 57.1×10^4 CFU/g, while fungal populations ranged from 1.67×10^4 CFU/g to 7.7×10^4 CFU/g (Table 2). Bacterial populations consistently outnumbered fungal populations across all samples, indicating the predominance of bacteria in the gut microbiota.

Table 2. Microbial Colony Counts (CFU/g) from the Gut of *Lambis lambis* on Nutrient and Potato Dextrose Agar Medium.

Microbial Colony Counts (CFU/g) from the Gut of <i>Lambs lambs</i> on Nutrient and Potato Dextrose Agar						
Homogenized Gut Samples	Replicate Samples	Per	Count Colonies	Average Colonies	Per	Mean CFU/g
Nutrient Agar						
Sample 1	R1		499	195		19.5x10 ⁴
	R2		34			
	R3		52			
Sample 2	R1		148	459		45.9 x10 ⁴
	R2		1,058			
	R3		171			
Sample 3	R1		940	571		57.1x10 ⁴
	R2		448			
	R3		324			
Potato Dextrose Agar						
Sample 1	R1		1	2		2 x10 ⁴
	R2		4			
	R3		1			
Sample 2	R1		2	77		7.7x10 ⁴
	R2		227			
	R3		1			
Sample 3	R1		1	1.67		1.67 x10 ⁴

Most Probable Number (MPN) of Coliform

The data showed that all *Lambis lambis* gut samples collected at Cowrie Island at Honda Bay, Puerto Princesa City, Palawan were positive for coliform bacteria, with MPN index ranging from 150 to 460 MPN per 100 mL (Table 3). All samples tested showed coliform counts which exceeded the recommend fecal coliform bacteria limit, which is 100 MPN per 100 mL with ‘Fair’ to ‘Poor’ classification of samples (Table 3).

Table 3. Most Probable Number (MPN) of Coliform and Bacteriological Classification of the Gut Samples of *Lambis lambis*.

Guts of <i>L. lambis</i> Sample (Triplicate)	MPN Index per 100 mL	Category (Grade)	Interpretation
Sample 1	150	II (fair)	Indicates moderate presence of coliform bacteria, suggesting exposure to contaminated environments.
Sample 2	240	III (fair)	Indicates increased bacterial contamination likely influenced by human activities.
Sample 3	460	IV (poor)	Indicates heavy contamination and potential risk to human health.

DISCUSSION

Microbial Abundance

The results reveal that *Lambis lambis* from Cowrie Island harbors a gut microbiota dominated by bacteria, with counts consistently exceeding fungal populations by approximately 10-fold. This bacterial dominance likely reflects the established metabolic roles of bacteria in digestion, nutrient assimilation, and energy metabolism in marine gastropods, consistent with previous findings in other mollusks (Nakagawa et al. 2017).

THEME: HEALTH, ENVIRONMENT, AND COMMUNITY WELL-BEING

The bacterial abundance may also indicate adaptation to the local environmental conditions and food composition in Honda Bay. In contrast, the presence of fungi in substantially lower and more variable amounts (ranging from 1.67×10^4 to 7.7×10^4 CFU/g) suggests they function as transient residents rather than permanent members of the core microbiota (Polinski et al. 2019). The variability in fungal counts across specimens could reflect differences in environmental exposure, dietary inputs, or individual host factors influencing colonization dynamics. These findings align with the general pattern observed in marine invertebrate microbiota, where bacteria comprise the dominant microbial community while fungi play secondary ecological roles.

Coliform Contamination

The detection of coliform bacteria exceeding the recommended standard limit of 100 MPN/100 mL in all ten specimens is a significant finding that warrants careful interpretation. The MPN values ranging from 150 to 460 MPN/100 mL indicate systemic exposure of *L. lambis* to fecal contamination, likely influenced by multiple anthropogenic stressors in Cowrie Island, including tourism activities, inadequate sewage treatment, coastal development, and improper waste disposal (Cayabo et al. 2021; Rabia et al. 2023). These elevated coliform levels indicate not only environmental pollution but also potential public health risks for consumers of *L. lambis* harvested from these waters, as coliforms are established indicators of fecal contamination and the potential presence of pathogenic microorganisms. This finding underscores the need for regular microbiological monitoring of seafood resources in recreational waters.

Bioindicator Potential and Ecological Implications

This study provides empirical evidence supporting the use of *L. lambis* as a bioindicator species for assessing microbial pollution in coastal marine ecosystems. The gut microbiota of marine invertebrates serves as a biological archive of their environmental exposure, reflecting the microbiological quality of their habitat through both the presence of environmental bacteria and the accumulation of fecal contaminants (Wilkins et al. 2019). As a sedentary or slow-moving benthic organism with a filter-feeding or detritivorous feeding strategy, *L. lambis* integrates microbial signals over time, making it particularly valuable for assessing chronic or spatial patterns of microbial pollution that point-source water sampling may miss. The consistent detection of coliform contamination across all specimens suggests that the Cowrie Island maybe exposed to fecal pollution, indicating that environmental remediation efforts and improved sanitation infrastructure are needed to protect both the ecosystem health and the food security of local fishing communities. Given the coliform levels detected in *L. lambis* specimens, consumers of this species should adopt rigorous food safety practices, including thorough cooking at temperatures sufficient to eliminate fecal indicator bacteria and potential pathogens, as well as proper handling and storage protocols. These preventive measures are essential to mitigate foodborne disease risks associated with the consumption of shellfish from potentially contaminated marine environments. Further investigation of the microbial composition of *L. lambis* and correlation with water quality parameters (e.g., dissolved oxygen, nutrient levels, bacterial community structure in water samples) would strengthen the bioindicator framework and enable more targeted management interventions in Honda Bay.

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GENERATIVE AI STATEMENT

AI (Artificial Intelligence) was used solely for language editing, grammar correction, sentence refinement, and formatting of the manuscript in accordance with the required publication template. All data collection, analyses, interpretations, and scientific conclusions were conducted and verified solely by the author.

ETHICAL CONSIDERATIONS

Collection and handling of *Lambis lambis* specimens were conducted following institutional and standard ethical guidelines for marine organism research. Specimens were handled using appropriate laboratory safety and aseptic procedures to minimize contamination and unnecessary harm during sample processing.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no competing interest associated with this study.

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ROLE OF ATUHORs: ECR conceptualized the study, conducted field sampling and laboratory analyses, interpreted the data, and prepared the initial manuscript. AYM supervised the laboratory procedures and revised the manuscript. JAMO provided overall conceptualization, reviewed and revised the manuscript.

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