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Microplastics in Indian squid (*Uroteuthis duvaucelii*) in region 1, Philippines

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ABSTRACT

Microplastic pollution threatens marine ecosystems and human health through seafood consumption in the Philippines. This study assessed the prevalence, abundance, and morphology of microplastics in the gastrointestinal tracts of Indian squid (*Uroteuthis duvaucelii*) from two major Bureau of Fisheries and Aquatic Resources - National Stock Assessment Program (BFAR-NSAP) landing sites in Region 1: Tubod, Sto. Tomas, La Union, and Port Sual, Pangasinan. Six specimens (n=3/site) underwent gastrointestinal tract dissection, 10% potassium hydroxide (KOH) digestion at 80°C for 48 hours, density separation, vacuum filtration, Nile red staining, and fluorescence. Prevalence reached 66.67% at Tubod (2/3 samples positive; up to 7 fragments and 6 fibers per specimen) versus 33.33% at Sual (1/3 positive; 3 mixed particles), with non-significant spatial differences (Fisher's exact test, $p=0.52$) attributable to limited sample size ($n=6$). Fragments (50% or total particles; irregular, jagged shapes) and fibers (50% of total particles; linear morphology) dominated, all exhibiting <20 µm and intense orange fluorescence indicative of synthetic polymers and consistent spectral emission. Higher abundances at urban-proximal Tubod implicate riverine runoff, fishing gear microfiber shedding, and post-harvest contamination despite shared Lingayen Gulf fishing grounds, aligning with neritic squid vulnerability via contaminated prey. These baseline data highlight food safety risks from secondary microplastics and support SDG 14 (Life Below Water). Recommendations include routine BFAR-NSAP screening with expanded n≥10/site, µ-Raman spectroscopy for polymer identification, riverine plastic booms, gear audits, and Local Government Unit-led waste interventions at high-risk sites.

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INTRODUCTION

Microplastics (plastic particles <5 mm) enter marine ecosystems from degraded debris, microbeads, and synthetic fibers via rivers, wastewater, and coastal activities worldwide (Hidalgo-Ruz *et al.*, 2012). These particles adsorb toxins, causing oxidative stress and posing health risks through seafood consumption (Rochman *et al.*, 2019). Cephalopods like squid, key fishery species, ingest microplastics via contaminated prey, with global studies detecting particles in edible tissues (Bothma *et al.*, 2024; Daniel *et al.*, 2020).

In the Philippines, coastal urbanization amplifies microplastic pollution, with detections in fish (up to 2 particles/specimen) (Benaires *et al.*, 2025), gastropods, and mussels (Bilugan *et al.*, 2021). Indian squid, comprising 90% of Philippine cephalopod catch, inhabits neritic waters (≤170 m) and feeds on contaminated crustaceans/fish (Legaspi *et al.*, 2024). Lingayen Fishing Gulf supports productive squid fisheries but faces pollution from industry/agriculture (Reichardt *et al.*, 2007).

Despite national surveys, no studies assess microplastics in Indian squids from Region 1 BFAR-NSAP sites- Tubod, Sto. Tomas, La Union and Port Sual, Pangasinan - despite their urban proximity and shared fishing grounds. This gap hinders food safety assessments and biodiversity monitoring in Philippine cephalopod fisheries, threatening SDG 14 (Life Below Water).

This study specifically aimed to (1) determine the prevalence of microplastics, (2) quantify microplastic particles, and (3) characterize their morphology in Indian squid collected from major landing sites in Region 1, Philippines.

MATERIALS AND METHODS

Study area

This study focused on two primary National Stock Assessment Program (NSAP) landing sites in Region 1, Philippines (Fig. 1): Tubod Fish Port in Sto. Tomas, La Union (16°15'03.78"N 120°23'57.35"E) and Port Sual Fish Port in Pangasinan (16°04'00.15"N 120°05'50.60"E), both situated along the western Lingayen Gulf coastline. Indian squid samples were collected from catches originating from shared offshore fishing grounds approximately 10-20 nautical miles from shore within Lingayen Gulf, a productive semi-enclosed embayment supporting regional cephalopod fisheries.

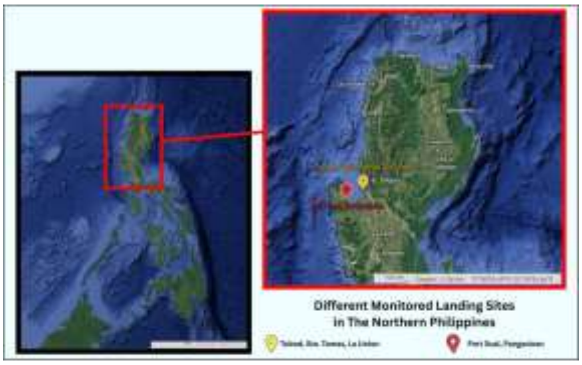

Fig. 1. Map of BFAR-NSAP identified sampling sites in Lingayen Fishing Gulf (Google Earth)

Experimental design

This cross-sectional study analyzed six (6) Indian squid (3/site × 2 NSAP sites: Tubod, Sto. Tomas, La Union and Fishport Sual, Pangasinan), randomly selected via fishbowl method from 10 specimens/site.

Collection procedure

Ethical standards were observed through seeking permission from authorities to ensure smooth collection of data and gather samples in specific areas. The biota sampling and preparation of samples was adopted from the procedures described by AMAP Litter and Microplastics Monitoring Guidelines (Version 1.0, 2021) cited by Onda *et al.* (2021).

Quantification of microplastics in samples was adopted from the procedures described by Onda *et al.* (2021). Characterization of microplastics was adopted from Harshvardhan and Jha (2013); Jung *et al.* (2018), Onda *et al.* (2021) and Hidalgo-Ruz *et al.* (2012). The use of appropriate Personal Protective Equipment (PPE) during laboratory procedures to safeguard against potential hazards associated with handling biological samples.

Biota sampling and preparation

After the collection, the samples underwent a meticulous washing process using pre-filtered water to eliminate any external contaminants. Each sample was carefully wrapped in aluminum foil after washing and secured with rubber bands to prevent further contamination during transportation. These wrapped samples were stored in an ice box and promptly transferred to a -20°C freezer until lab processing (AMAP Litter and Microplastics Monitoring Guidelines, Version 1.0, 2021. cited by Onda *et al.*, 2021).

Extraction of microplastics from samples

The extraction of microplastics from the samples began with thawing and thoroughly rinsing their exterior to minimize particle contamination during processing. The entire gastrointestinal tract of the sample organism was examined to accurately estimate ingested microplastics. Specifically, the esophagus was cut while keeping the entire stomach intact, and the gut was cut approximately 2-3 mm before the anus. The weight of the stomach and gut were recorded. The excised stomach and gut were placed in a clean 1L beaker, and 10% KOH solution, three times the tissue volume, were added to facilitate digestion. The beaker opening was covered with foil, and the setup was maintained at 80°C for 48 hours, with manual agitation every 12 hours to ensure thorough digestion (Onda *et al.*, 2021).

Density separation was performed by adding 150 mL of saturated sodium chloride (NaCl) solution to the digested solution and mixing well if residues were observed to remain digestate. The mixture was allowed to settle overnight, which enabled the separation of denser materials. Once the tissues are fully digested, microplastics were extracted using vacuum filtration. Filtration was conducted with 20-µm quantitative ashless filter paper in a Buchner funnel and flask setup, and the filter paper was stored in a sterile petri dish to avoid contamination (Onda *et al.*, 2021).

Most importantly, to digest any residual organic matter on the filter, 2-3 drops of 30% hydrogen peroxide (H_2O_2) were added. The filters were then dried in an oven at 60°C overnight. The petri dish containing the dried filter was wrapped in foil and stored until done with the microplastic quantification (Onda *et al.*, 2021).

Quantification and visualization of microplastics

In the quantification of the microplastics, the researcher dropped around 600 µL of Nile Red (NR) working solution onto each filter paper containing the particles, then carefully ensured that all surface has been covered with the solution. Later, the petri dish was enclosed with aluminum foil and allowed to rest for 24 hours for excess moisture to evaporate. Each sample was placed under the microscope stage. It was observed through an orange filter in a shine blue light. A photo was taken and the fluorescing microplastics was counted (Onda *et al.*, 2021).

Characterization of microplastics

Microplastics were characterized by visual morphology according to their type, shape and color, following the guidelines established by Harshvardhan and Jha (2013); Jung *et al.* (2018), Onda *et al.* (2021) and Hidalgo-Ruz *et al.* (2012). FT-IR spectroscopy was not performed due to particle size <20 µm.

Statistical analysis

Prevalence was compared between sites using Fisher's exact test ($\alpha = 0.05$) in R v4.3.1 (R Core Team, 2023). Frequency, percentage, and descriptive analyses summarized quantity and morphology (Onda *et al.*, 2021).

RESULTS AND DISCUSSION

Microplastics contamination across coastal locations

Assessment on the prevalence of microplastic contamination in Indian squid collected from major landing sites monitored by the National Stock Assessment Program (NSAP) across Region 1. showed varying contamination rates. The catches originated from the same offshore areas of Lingayen Gulf fishing ground, yet the percentage of seafood samples containing microplastics varied across landing sites. Fig. 2 shows 66.67% prevalence in Tubod, Sto. Tomas, La Union, while Port Sual, Pangasinan demonstrated 33.33% presence of microplastics.

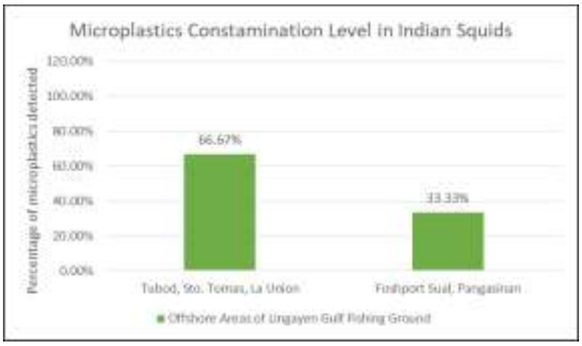

Fig. 2. Percentage of Indian squid containing microplastics from selected monitored landing sites

Higher prevalence in Tubod, Sto. Tomas, La Union reflects its closer proximity to urban centers and intensive fishing activity channeling plastic waste via riverine inputs to offshore Lingayen Gulf areas (Pustadan, 2024). The lower prevalence at Port Sual, Pangasinan corresponds to relative differences in nearshore anthropogenic sources despite shared fishing grounds. These spatial patterns, despite identical catch origins, align with neritic squid vulnerability to suspended microplastics and potential post-harvest contamination differences (Rochman *et al.*, 2019).

Statistical analysis of prevalence

Prevalence varied across sites: 66.67% (2/3 samples) in Tubod, Sto. Tomas, La Union versus 33.33% (1/3 samples) in Port Sual, Pangasinan, despite catches from identical offshore Lingayen Gulf fishing grounds. Fisher's exact test compared contamination rates (Tubod: 2 positive/1 negative vs Port Sual: 1 positive/2 negative), yielding $p=0.52$. This non-significant result ($p>0.05$) reflects limited statistical power due to small sample size ($n=6$ total), consistent with the study's baseline characterization objectives.

These results indicate regional variations in marine pollution levels, shaped by coastal development, waste management strategies, and the closeness to human activities. The presence of microplastics in seafood sourced from both relatively untouched and urbanized landing areas underscores the pressing necessity for unified efforts to mitigate marine plastic pollution. In conclusion, this research offers significant baseline data essential for advancing Sustainable Development Goals 3 (Good Health and Well-being), 12 (Responsible Consumption and Production), and 14 (Life Below Water), amidst the ongoing challenge of microplastic contamination.

Characteristics and quantity of microplastics present in selected seafood species analyzed using Nile red staining

The analysis was conducted using Nile red staining, a rapid and reliable method for identifying synthetic polymers in biological samples. Nile red selectively binds to hydrophobic materials such as plastics and under fluorescence microscopy, produces an intense orange glow, which allows clear distinction from organic particles (Maes *et al.*, 2017). This technique provided a consistent means to detect and evaluate the quantity and morphological characteristics of microplastics in different locations.

Table 1. Characteristics and quantity of microplastics detected in Indian squid from selected monitored landing sites

Location	Sample code	Quantity detected	Characteristics of microplastics		
			Type	Shape	Color
Pantal, Dagupan City, Pangasinan	1	7	Fragment (7)	Irregular (7)	Intense Orange
	2	6	Fiber (6)	Line (6)	Intense Orange
Port Sual, Pangasinan	2	3	Fragment (1), Fiber (2)	Irregular (1), Line (2)	Intense Orange

Quantity of microplastics

The results (Table 1) showed varying levels of microplastic contamination in Indian Squid samples depending on the collection site. The highest concentrations were recorded in samples from Sto. Tomas, La Union - particularly from Barangay Tubod - with individual samples containing up to 7 and 6 microplastics, respectively, while Port Sual, Pangasinan yielded 3 particles in the positive sample. These variations may be attributed to Indian squid's neritic ecology in offshore Lingayen Gulf fishing grounds, making them susceptible to suspended microplastics, with site differences possibly arising from post-harvest contamination or localized runoff (Onda *et al.*, 2021).

The notably higher counts Tubod reflects local coastal pollution sources including urban proximity, fishing activities, and Lingayen Gulf tributary inputs (Pustadan, 2024). These findings align with cephalopod vulnerability to microplastic ingestion via contaminated prey in productive coastal waters (Rochman *et al.*, 2019; Daniel *et al.*, 2020).

Characteristics of microplastics in terms of type, shape, and color

Microplastic particles extracted from Indian squid gastrointestinal tracts measured <20 µm, rendering them undetectable via available FTIR-ATR spectroscopy and necessitating reliance on visual morphology coupled with Nile red fluorescence validation (Maes *et al.*, 2017). This methodological constraint notwithstanding, comprehensive microscopic examination yielded robust morphological profiles: fragments comprised 50% of total particles (8/16 across positive samples), exhibiting jagged irregular shapes diagnostic of secondary microplastics derived from macroscopic plastic degradation through UV weathering, mechanical abrasion, and biofouling fragmentation (Fig. 3; Hidalgo-Ruz *et al.*, 2012). Fibers accounted for the remaining 50% (8/16), displaying uniform linear morphology with parallel margins characteristic of synthetic textile microfibers shed from fishing nets, ropes, and laundry effluents prevalent in Lingayen Gulf fishing operations (Fig. 4).

All 16 particles demonstrated intense orange fluorescence under Nile red staining (blue light excitation, Olympus CX43 with orange emission filter), unequivocally confirming their anthropogenic polymeric composition through selective adsorption to hydrophobic domains absent in biogenic debris (Maes *et al.*, 2017; Onda *et al.*, 2021). Quantitative color analysis revealed consistent spectral across fragment and fiber types, with no false positives among screened fields-of-view. Fragments predominated in Tubod samples (100% of sample 1's 7 particles), likely reflecting accumulation of degraded packaging/buoyant debris within squid's neritic feeding zone, while fibers were ubiquitous across sites (Tubod sample 2: 6 particles; Port Sual: 2/3 particles), implicating gear-related inputs during intensive Lingayen Gulf harvests (Hantoro *et al.*, 2019).

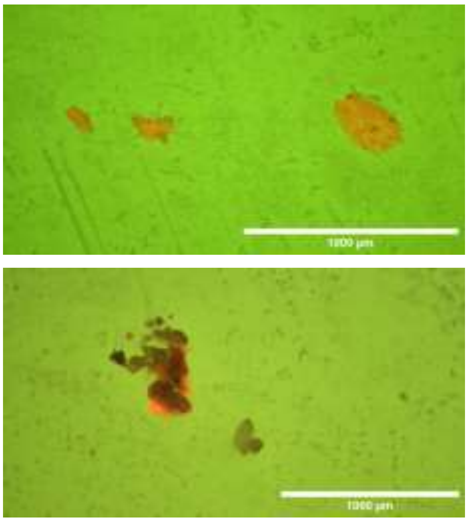

Fig. 3. Fragment-type microplastics (irregular-shape, 100µm scale bar) under Nile red staining, Olympus CX43 fluorescence microscope

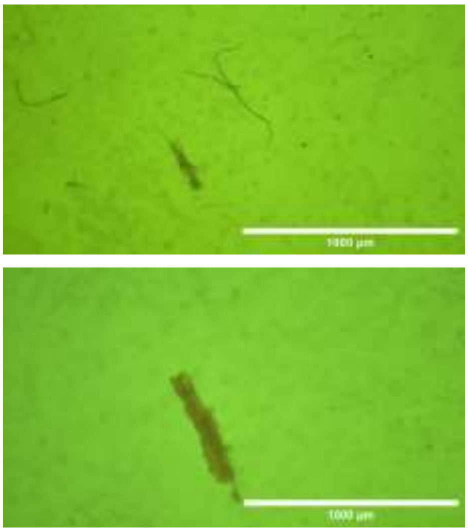

Fig. 4. Fiber-type microplastics (linear-shape, 100µm scale bar) under Nile red staining, Olympus CX43 fluorescence microscope

These type-shape distributions mirror global cephalopod contamination profiles- where fragments/fibers constitute 70-90% of gastro intestinal tract microplastics in loliginid squids- while the <100 µm size fraction underscores bioavailability to predators including humans via incomplete evisceration during food preparation (Daniel *et al.*, 2020; Bothma *et al.*, 2024). The fibrous fraction particularly alarms, as high surface-area-to-volume ratios enhance persistent organic pollutant adsorption, amplifying trophic transfer risks through squid consumption patterns in Region 1's coastal communities (Rochman *et al.*, 2019).

Methodological rigor via triplicate staining/counting protocols ensures result reliability despite spectroscopic limitations, establishing baseline morphological signatures for future polymer-specific analyses via µ-Raman or pyrolysis-GC/MS.

CONCLUSION

This study provides baseline evidence of microplastic contamination in the gastrointestinal tracts of Indian squid (*Uroteuthis duvaucelii*) collected from major NSAP landing sites in Region 1, Philippines, demonstrating that microplastics are already present in commercially important cephalopod species. The findings revealed a higher prevalence and abundance of microplastics in specimens from Tubod, Sto. Tomas, La Union compared with Port Sual, Pangasinan, although the difference was not statistically significant due to the limited sample size. Fragments and fibers were the dominant particle types, indicating that both degraded plastic debris and synthetic fishing-related materials are likely sources of contamination in the Lingayen Gulf fishing grounds. These results highlight the vulnerability of neritic squid to microplastic ingestion through contaminated prey and surrounding waters, emphasizing potential ecological and food safety implications for coastal communities that rely on squid as a dietary resource. Overall, the study contributes valuable preliminary data for marine pollution monitoring and underscores the need for expanded sampling, improved waste management, and continued research to better understand the extent and impacts of microplastic contamination in Philippine marine fisheries.

RECOMMENDATIONS

Future studies should expand the sample size and sampling frequency across multiple landing sites in Region 1 and other Philippine coastal areas to improve statistical power and provide a more representative assessment of microplastic contamination in Indian squid (*Uroteuthis duvaucelii*). Advanced analytical techniques such as µ-Raman spectroscopy or FTIR spectroscopy should be incorporated to accurately identify polymer types and determine potential sources of microplastics. Routine monitoring of microplastics in commercially important seafood species should also be integrated into national fisheries and environmental monitoring programs, particularly through agencies such as BFAR and local research institutions. In addition, coastal waste management strategies should be strengthened, including improved plastic waste collection, reduction of single-use plastics, and proper disposal practices in fishing communities and urban coastal areas. Awareness campaigns targeting fishers, seafood vendors, and coastal residents should also be implemented to promote responsible waste handling and reduce plastic leakage into marine ecosystems. Collectively, these measures will support long-term monitoring of marine pollution, help protect seafood safety, and contribute to the conservation of marine biodiversity in the Philippines.

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