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Susceptibility of *Sardinella lemuru* to emerging marine microplastic pollution

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ABSTRACT

Marine microplastics are emerging pollutants that impact across levels of marine food chain at a global scale. Its presence was determined on *Sardinella lemuru*, a commercial pelagic fish that are harvested generally in the Northern Mindanao, consumed locally, and exported worldwide as bottled or canned sardine products. The stomach contents of 600 sardines were examined visually under a microscope, stained with Rose Bengal, and tested with hot needle technique to identify ingested microplastics. These anthropogenic particles were measured and physically classified into fibers, fragments, and films. Results of this study showed that 85% of *S. lemuru* were already contaminated with 3.74 ± 3.92 # of microplastics even before being processed into various sardine products. These microplastics ranged from 0.12 to 21.30 mm and 80 % were mostly < 2.5 mm size classes. The dominant microplastics were 97.94 % in the form of fibers while 1.52 % and 0.54 % were respectively classified into fragments and films. Method validation by isolating microplastics from spiked samples (n = 30) with three retrieval attempts showed 100% recovery efficiency. While results from Canonical Correspondence Analysis of ingested microplastic data had no relationship with the standard lengths of the sardine and the masses of ingested food materials at varying size classes, the total number of ingested microplastics from 2014 to 2016 were directly correlated (r^2=0.91, p=0.003) with the human population at the landing sites along the coastline of northern Mindanao.

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INTRODUCTION

Marine microplastics are ubiquitous anthropogenic particles less than 5 mm which may be found throughout the polar regions, equator, coastal- pelagic zones, and in the abyssal sediments (Cole *et al.*, 2011; Crawford and Quinn, 2017; Free *et al.*, 2014; Waller *et al.*, 2017). Some of these microplastics such as the microbeads, glitters, and plastic pellets were produced intentionally while others resulted from the degradation of larger plastic litters. Regardless of the microplastic type, when unmanaged, these synthetic particles will potentially end up in the municipal wastewater, freshwater systems, and eventually in the ocean (Anderson *et al.*, 2016; Avio *et al.*, 2017). Barnes (2005) reported that the amount of anthropogenic marine plastic debris in the southern hemisphere is directly proportional to the increase in human population per 10 degrees latitude. Jambeck *et al.* (2015) ranked the Philippines as the third country to mismanage plastic waste and has been estimated to produce 0.28 – 0.75 MMT/year of marine plastic debris. The North Pacific Ocean, located in the east seaboard side of the Philippines, contains 116 x 10^{10} pieces of marine microplastic within the size class of 1 - 4.75 mm followed by 68.8 x 10^{10} pieces in the size class of 0.33 – 1 mm (Eriksen *et al.*, 2014). Whether or not the Philippines is a major sink or source of microplastics, the marine biodiversity in the entire archipelago is currently under threat. Microplastics are ingested by aquatic organisms, including corals, barnacles, sea cucumbers, polychaete worms, zooplankton, rotifers, ciliates, crustaceans, amphipods, mollusks and fishes (Chae and An, 2017; Cole *et al.*, 2011; Wright *et al.*, 2013). Upon ingestion, it releases plasticizers and can physically puncture or obstruct the digestive tract of an organism which may eventually lead to death (Wright *et al.*, 2013). Various marine communities are threatened with unpredictable ecological effects due to bioaccumulation and biomagnification of microplastics across the trophic levels in the marine food chain (Avio *et al.*, 2017; Chae and An, 2017). As some of these critical marine resources are important for human consumption, it becomes inevitable to be at risk from the threats posed by the emerging marine microplastic pollution. Karami *et al.* (2017) reported that canned sardines and sprats from Japan, Iran, Latvia, Poland, Scotland, and Russia are already contaminated by micro- and mesoplastics. Using Micro-Raman spectroscopy, the most abundant plastic polymers they found in the sardine products are polypropylene (PP) and polyethylene terephthalate (PET). They also deduced that the presence of micro- and mesoplastics in canned sardines and sprats might be due to translocation of these particles into the edible tissues, improper gutting or contamination from the canning process. However, in cases where cutting edge technology is not available to characterize the plastic polymers, microscopic visual inspection while using Rose Bengal Biological stain and employing hot needle test are sufficient enough to identify microplastic particles in fish samples (Davison *et al.*, 2011; Kosuth *et al.*, 2018; Vendel *et al.*, 2017). In the Philippines, sardines are important marine food resource and are mainly harvested from the Northern Mindanao particularly in the offshore waters of Zamboanga Peninsula. About 50-60% of the total annual production are harvested from this major fishing ground and processed into bottled or canned sardines among others (Rola *et al.*, 2018). The sardine catch landings are generally dominated by *Sardinella lemuru*, which was previously misclassified as *S. longiceps* (Willette and Santos, 2013). Since the occurrence of microplastics has major implication particularly on food safety, there is a dire need, therefore, to determine whether a species of commercial value is already ingesting microplastic. Hence, the main objective of this study is to determine the susceptibility of *S. lemuru* to microplastic pollution through visual inspection of stomach content stained with Rose Bengal under the microscope while incorporating hot needle test to verify microplastic particles. Specifically, the study aims are to 1) determine the microplastic morphotype and size spectra which sardines are likely to ingest, 2) explore biological variables such as sardine standard length and size of classified food type as factors for microplastic ingestion, and 3) to relate the cumulative ingested microplastics in sardines to human population in Butuan, Dapitan, Dipolog, Gingoog, Illigan, Macajalar, Patawag, and Sindangan landing sites in the Philippines during the spawning seasons from 2014 to 2016.

MATERIALS AND METHODS

Sardine collection

Sardines were collected during the spawning seasons from 2014 to 2016 from various landing sites across Northern Mindanao namely: Butuan, Dapitan, Dipolog, Gingoog, Illigan, Macajalar, Patawag, and Sindangan (Fig. 1). These landing sites are immediately located to each corresponding fishing grounds. The

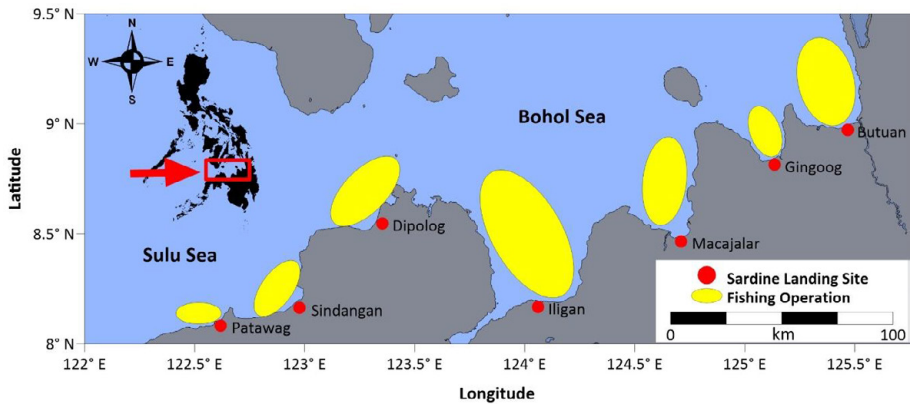

Fig. 1: Geographic location of the study area along with the sampling location of sardines from landing sites across Northern Mindanao in Philippines

total area of the municipal waters for fishing considered in the study is approximately 10,160 km^2. A total of 600 sardines was used in this study secondary only to various biological investigations such as molecular and microscopic identification of gut content, chlorophyll a and phaeopigment characterization, size classed dry mass food content, and endoparasitic surveys. Five individual sardines were randomly sorted out for each of the mentioned gut content studies. Summary of sardine samples used in this study is shown in Table 1.

Standard length and wet body mass determination

From the fishing ground, the collected sardines were initially stored in a cooler filled with ice, kept frozen overnight, and shipped to the laboratory the following day. Sardines were thawed before wet body mass (± 0.01 g) was measured using OHAUS CL Series in the laboratory. Standard length (± 0.65 mm) was determined from the digitally calibrated photo of each sardine using ImageJ (v1.50, National Institutes of Health, U.S.A.). The sardines in this study were identified by molecular technique as *S. lemuru* (Labrador *et al.*, 2019).

Stomach and microplastic extraction

Each sardine was carefully dissected with a surgical scalpel to extract the stomach (Garvey and Chipps, 2013). If not processed immediately, stomachs were stored individually in a glass culture tube with 90% ethanol and kept in the freezer. Only those stomachs that appeared full were included in the study. The stomachs were all cleaned with 9% saline solution, transferred to a sterile petri dish, and dissected to extract all the ingested food particles. For microplastic identification, Rose Bengal biological stain was used following Kosuth *et al.* (2018). The stomach contents were rinsed into a gridded petri dish, stained with Rose Bengal solution (200 mg/L, Sigma-Aldrich 95%), and visually isolated microplastics onto a Sedgewick Rafter counting chamber using micro dissecting tungsten needle and a fine tip tweezer under a Nikon SMZ-1 dissecting stereomicroscope with 2x auxiliary lens. Microplastics were isolated at 3 recovery attempts per sample. The counting chamber with microplastic samples was transferred to a digital microscope (DinoLite with DinoCapture 2.0) to photopgraph and measure (± 0.05 mm) the longest dimension of every

Table 1: Sampling frequency and number of sardines used in this study

Period		Bays						
Year	Month	Butuan	Dipolog	Gingoog	Iligan	Macajalar	Patawag	Sindangan
2014	Jul	26	-	26	-	20	-	-
	Aug	-	22	-	-	-	20	16
2015	Jan	25	-	25	20	30	-	20
	Jul	20	20	20		20	20	20
2016	Aug	20	12	20	20	20	-	18
	Nov	20	20	20	-	20	20	20

microplastic. Unstained particles were considered as microplastic and hot needle test was used for further confirmation. Microplastic particle would melt or be deformed when in contact with the hot needle tip (Baalkhuyur *et al.*, 2018). A total of 134 sardine samples designated for dietary analysis by microscopy was used to count, measure, and physically classify the isolated microplastic into fiber, fragment, and film morphotypes (Vendel *et al.*, 2017). Additional 466 sardine samples were used only for microplastic enumeration and physical classification following the Rose Bengal staining and hot needle test techniques under the dissecting stereomicroscope.

Stomach content size classification

After microplastics were fully recovered from samples that were assigned for measuring the dry mass of ingested food particles, we rinsed the stomach contents with Milli-Q water through a wet serial Nytal sieves of 20, 64, 100, 250, and 500 µm mesh sizes. Stomach content per size class was transferred to a pre-dried and pre-weighed 25mm GF/C filters and oven dried for 48-72 hrs at 60°C to determine the final and constant dry mass (± 0.1 mg) using Ohaus PA214 analytical balance (Garvey and Chipps, 2013).

Microplastic retrieval efficiencies

We performed a blind test to determine the retrieval efficiencies of microplastics following the approach of Budimir *et al.* (2018) with some modifications. About 30 microplastics were isolated and kept in scintillation vials with 10 ml 70% ethanol for every size class (<0.5, 0.5-1, 1-1.5, 1.5-2, and 2-2.5 mm). A pooled mixture was obtained by subsampling 5 ml from each vial into a glass culture tube. This mixture was used to spike a microplastic-free stomach content and retrieval of microplastics was performed each time with 3 recovery attempts following the staining and isolation of microplastic protocol as previously mentioned. We also kept a microplastic-free stomach content as a control sample. This procedure was performed routinely once a week and repeated for 30 times. We made a digital cell counter in MS Excel and hid the scores on a separate sheet. Scores of microplastics were accounted and compiled only at the end of the entire experiment. We found that the remaining microplastics were 16, 19, 15, 13, 16 pieces respectively for <0.5, 0.5-1, 1-1.5, 1.5-2, and 2-2.5 mm size classes at the end of the blind test. About 71 microplastics were accounted and used to spike the microplastic-free stomach content sample. Percent recovery efficiency (RE) for every microplastic retrieval attempt was estimated using the formula RE = (# of recovered microplastic / # of spiked microplastic) x 100.

Quality control

To avoid contamination during sample processing, cotton clothing covered with a cotton lab gown and a pair of nitrile powder free gloves were worn throughout the laboratory procedure. The lab space was vacuumed and disinfected with ethanol >30 mins before the actual sample processing, and all liquid substances used were pre-filtered with GF/C. We set up microplastic traps using 2 glass slides placed on the microscope stage and 10 more were randomly distributed around the workspace for monitoring purposes. These slides were inspected under the stereomicroscope in between sample processing. All dissecting materials, counting chambers, and petri dishes were rinsed with Milli-Q water, air dried, and visually inspected before use. We also kept and inspected blank vials, culture tubes and other sampling glassware. Both the dissecting stereomicroscope and digital microscope were hooded with autoclavable polypropylene bag to prevent possible microplastic fallout during sample inspection.

Data analyses

Canonical Correspondence Analysis (CCA) was used for data mining to evaluate the relationship between microplastic size classed data and its explanatory variables: fish standard lengths, ingested food particles. All data from 2014 to 2015 were transformed into log (n + 1), axis scored were centered and standardized to unit variance, axes scaled to compromise representation of both datasets, and scores for graphing microplastic size classes per bay were set as linear combination of the explanatory variables. Monte Carlo permutation procedure (999 permutations with 4029 random number seeds) was implemented to test the hypothesis of no relationship between the microplastic data and the explanatory variables at $p < 0.05$. Analyses were performed in PC-ORD Version 7.07 program (McCune and Mefford, 2018). To determine the relationship of ingested microplastic with human population, we performed regression analysis between the total number of ingested microplastic data from 2014 to 2016 and the human population in each landing site using census

data of 2015 acquired from the Philippine Statistics Authority (2019). Since microplastic data from Iligan Bay was poorly represented with only 2 sampling periods, it was excluded in the regression analysis that was performed in Minitab Version 18.1 (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

About 85% of the examined 600 *S. lemuru* had microplastics in their stomach compared to 96 % in *Sardina pilchardus* (Renzi *et al.*, 2019) and 100 % in *S. fimbriata* (Hastuti *et al.*, 2019). We accounted a total of 2, 238 microplastic in which 97.94 % mostly existed as fibers while 1.52 % and 0.54 % were respectively classified into fragments and films. These varieties of microplastic isolated from the stomach of *S. lemuru* are shown in Fig. 2 and the relative distribution of microplastic morphotypes across the study sites is shown in Fig. 3. It was estimated that a sardine from Northern Mindanao region may contain 3.74 ± 3.92 (mean ± standard deviation) # of microplastics. Majority of these microplastic were recovered 97.5 % during the first retrieval attempt and only 1.6 % and 0.8 % were recovered respectively for the second and third attempts. These microplastic recovery efficiencies were comparable to the validation study performed with the spiked sample in the laboratory. Three retrieval attempts were enough to recover 100% of the spiked sardine samples in which recovery efficiencies of 99.53 %, 0.33%, and 0.14% respectively for the first, second, and third retrievals were determined. No microplastic was found in the control samples, microplastic slide traps, blank glass vials, and glass culture tubes. As Rose Bengal staining technique effectively helped distinguished microplastic from other food items in the stomach of sardines, it was unable to stain some particles of biogenic origin. These include the phytoplankton cell wall-frustules, coccoliths which are made of calcium carbonate, and exoskeletons of zooplankton arthropods that are composed of chitin material (Davison and Asch, 2011). Extensive experience in plankton research, ease in microdissection/manipulation under the microscope, and the application of hot needle test were also major factors considered for the high recovery efficiencies of microplastic in the present study.

The size range of microplastics during the validation experiment varied from 0.42 to 2.35 mm in length while those extracted from the field samples ranged from 0.12 to 21.30 mm. High recovery efficiencies of

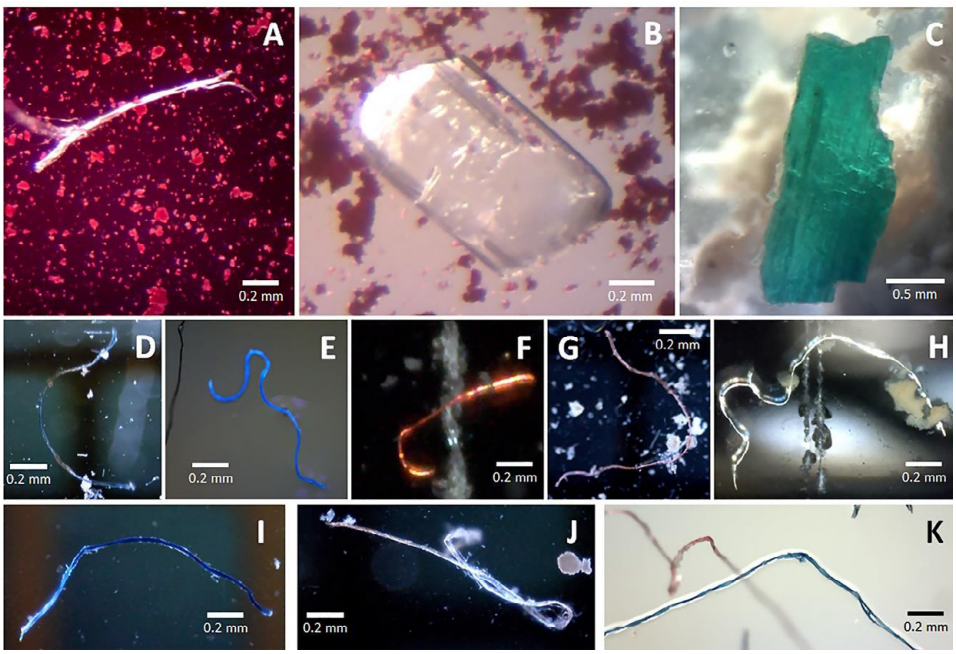

Fig. 2: Microplastics ingested by *S. lemuru*. (A-B) Microplastic and stomach content stained with 1% Rose Bengal. Microplastics isolated from the stomach of sardines were classified into (A, D-K) fibers, (B) fragments, and (C) films

Table 2: Summary of reported microplastic ingestion by sardines. (MP) Microplastic, (SL) Standard length, (TL) Total length, mean ± standard deviation, (min-max) range

Sardine Species	Site	Year	n	Fish length (cm)	% MP Ingestion in sardine	Total # of MP	Ave. # ingested MP/ind	MP min-max length (mm)	Total # MP fibers	Reference
Sardina pilchardus	English Channel	2013	20	23.7 (SL)	45	11	1.22	0.25-9.5	7	(Collard *et al.*, 2017)
	Portuguese coasts	2013	12	15-21 (SL)	0	-	0	-	-	(Neves *et al.*, 2015)
	Central Adriatic Sea, Italy	2013-14	80	14.23 (TL)	96	-	13.75 ± 11.15	0.6-8.5	110	(Renzi *et al.*, 2019)
	Spanish Western Mediterranean coasts	2015	105	16.91 (SL)	15	-	0.21 ± 0.23	-	-	(Compa *et al.*, 2018)
	Turkish Mediterranean Coast	2015	7	-	57	-	1.57	-	-	(Guven *et al.*, 2017)
	Gulf of Lions	2015	85	-	12	17	0.20 ± 0.69	-	-	(Lefebvre *et al.*, 2019)
Sardinops sagax	Chile	2016	7	18.1 ± 1.8 (TL)	0	-	0	-	-	(Ory *et al.*, 2018)
Sardinella fimbriata	Pantai Indah Kapuk coast, Indonesia	2015	10	-	100	-	20 ± 8	-	-	(Hastuti *et al.*, 2019)
Sardinella gibbosa	Northern Bay of Bengal	2017-18	25	-	100	80	3.20 ± 1.16	<0.5-2	44	(Hossain *et al.*, 2019)
Sardinella longiceps	Indian Coast	2010-12	10	-	60	-	-	0.5-3	-	(Sulochanan *et al.*, 2019)
Sardinella lemuru	Northern Mindanao, Philippines	2014-16	600	13.6 ± 1.1 (SL)	85	2238	3.72 ± 3.97	0.12-21.30	2192	**Present study**

microplastics were expected in the present study due to relatively coarser microplastic sizes encountered in the validation experiment and field sardine samples. In the study of Budimir *et al.* (2018), the recovery efficiency was only 84 ± 15% for microplastic size range of 100 µm to 1mm after tissue digestion protocol. This size range only accounted approximately 30% of the ingested microplastic in the present study. Our results suggest that *S. lemuru* was more susceptible to ingesting microplastic of size range <0.5 to 2.5 mm which accounted for a cumulative contribution of 80% (Fig. 4). Compared to *S. pilchardus*, which have been reported to ingest microplastic ranging from 0.25 to 9.5 mm (Collard *et al.*, 2017; Renzi, Specchiulli *et al.*, 2019), *S. lemuru* ingested a relatively wider size spectrum of microplastic. A comparative statistic of microplastic ingestion from other sardine species is presented in Table 2. Among many factors considered in various studies used to explain the ingestion of microplastic in fishes are length of fish and stomach content (Boerger *et al.*, 2010; Compa *et al.*, 2018; Halstead *et al.*, 2018).

We took a similar approach and used CCA primarily to explore whether the ingested microplastic of various size classes were related to the length and ingested food particles. The explanatory variables considered in this study showed some degree of independency to each other as shown in the pairwise correlation matrix in Table 3. The first two axes contributed a total of 29.3 % of the cumulative variance explained in microplastic data (Table 4). Most of the variance explained was in axis 1 (17.1%) followed by axis 2 (12.2 %) and axis 3 (7.2). The correlations and biplot scores of all the explanatory variables are shown in Table 5. The main factor with the highest correlation in axis 1 was the length of the sardine (-0.42) while in axis 2 was the sardine food item of 64 µm dry mass (-0.82). These two factors are evident in the CCA biplot in terms of their vector direction and length (Fig. 5). Consequently, due to low correlation values and cumulative data variance explained, the ordination of microplastic data by landing site overlapped in space defined by axes 1 and 2.

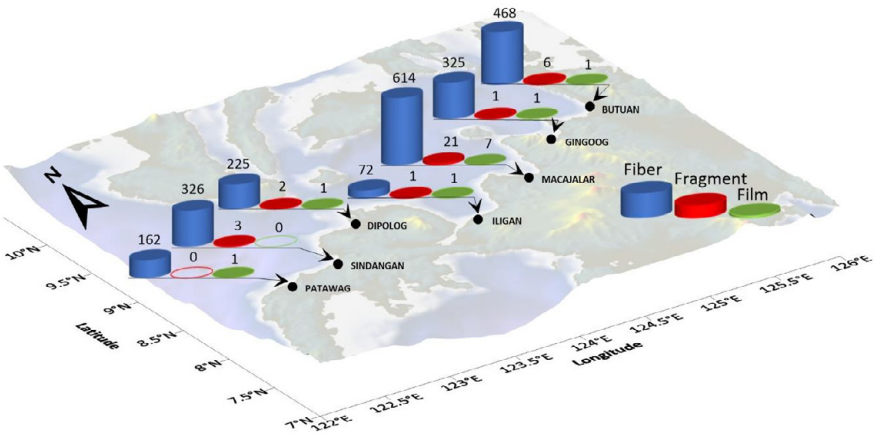

Fig. 3: Spatial distribution of the total microplastics classified into fiber, fragment, and film

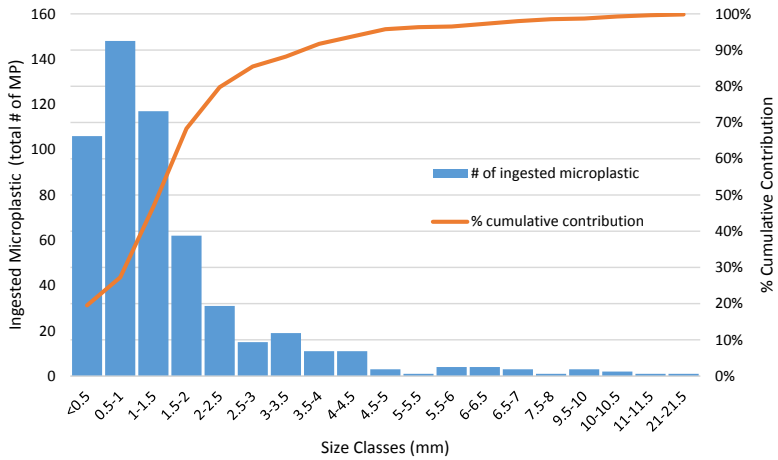

Fig. 4: Frequency of microplastic ingestion with corresponding percent cumulative contribution at various size classes in *S. lemuru* (n=134)

Furthermore, the hypothesis that there was no relationship between the microplastic data and the explanatory variables was accepted based on the low eigenvalues for both axes 1 (0.14) and 2 (0.1). These eigenvalues were within the limited range of 0.043 to 0.189 as expected by chance. Moreover, the correlation between microplastic data and its explanatory variables, particularly in axis 1, was not significant (p=0.105) during the randomization test in the Monte Carlo permutation procedure (Table 6).

In another study, fish body length is also not correlated to the amount of ingested microplastics in *S. fimbriata* and other commercial fishes in Indonesia (Hastuti *et al.*, 2019). Additionally, fishes found in Sydney Harbor have comparable amount of microplastic among species when standardized with gut content weight (Halstead *et al.*, 2018). While Boerger et al. (2010) reported that larger fish has more pieces of plastic in their guts than smaller fish, Compa *et al.* (2018) found that larger fish with better physical condition are less likely to ingest microplastics. It is apparent in the current study and those previously mentioned that fish morphology and food content do not immediately provide a direct explanation to the amount of ingested microplastic. As such, the density of bioavailable microplastic in the marine environment, especially those adjacent to the urban coastline, may be considered as an important factor among others. Particularly, Guven *et al.* (2017) reported that fish with high amount of

Table 3: Pairwise correlation matrix among the explanatory variables

	Correlations			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Length	-0.42	-0.119	0.702	-0.257	-0.067	0.346
500µm	0.23	-0.08	0.611	0.141	-0.045	0.301
250µm	0.326	-0.44	0.558	0.199	-0.248	0.275
100µm	-0.056	-0.486	0.323	-0.034	-0.274	0.159
64µm	-0.057	-0.82	0.193	-0.035	-0.461	0.095
20µm	-0.002	-0.256	-0.597	-0.001	-0.144	-0.294

Table 4. CCA axis summary statistics

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.14	0.1	0.059
Variance in microplastic data			
% of variance explained	17.1	12.2	7.2
Cumulative % explained	17.1	29.3	36.5
Pearson Correlation	0.92	0.877	0.892
Kendall (Rank) Correlation	0.809	0.765	0.471

ingested microplastics are found in areas with high amount of microplastics in seawater and sediment. Moreover, the amount of microplastic in the sediment is directly related to the human population density in major urban coastline of the world (Browne *et al.*, 2011). In our present study, the size of human population was also evident as a critical factor to account for microplastic ingestion in *S. lemuru*. We found that the total amount of ingested microplastics was significantly correlated (r^2=0.914, p=0.003) to the size of human population in the landing sites (Fig. 6). The highest amount of ingested microplastic was found in Macajalar Bay while the least was recorded in Patawag Bay. Fig. 7 is the summary statistics of ingested microplastic in *S. lemuru* per landing site.

Since baseline studies are very limited locally, the monitoring and assessment of microplastics in the water, sediment, prey items, and in other economically important marine species are highly recommended in order to determine their extent of vulnerability to microplastic pollution. There is a possibility that the reported microplastics in the present study was underestimated as we did not employ full digestion of gut content and that microplastics in the prey of *S. lemuru* were unaccounted. We plan to do a comparative study between the digestion approach and the current

Table 5. Correlation and biplot scores of the 6 explanatory variables

	Length	500µm	250µm	100µm	64µm	20µm
Length	1					
500µm	0.5528	1				
250µm	0.4822	0.8341	1			
100µm	0.4203	0.6652	0.8076	1		
64µm	0.4138	0.3737	0.5597	0.6968	1	
20µm	-0.0506	-0.1303	0.1352	0.3507	0.3258	1

Table 6. Monte Carlo test results for eigenvalues and species-environment correlations based on 999 runs with randomized data

		Randomized data			
Axis	Real data	Mean	Minimum	Maximum	p
	Eigenvalue				
1	0.14	0.107	0.061	0.189	0.047
2	0.1	0.075	0.043	0.122	
3	0.059	0.054	0.029	0.087	
	Microplastic-Sardine length and food correlations				
1	0.92	0.854	0.690	0.983	0.105
2	0.877	0.845	0.633	0.985	
3	0.892	0.800	0.561	0.976	

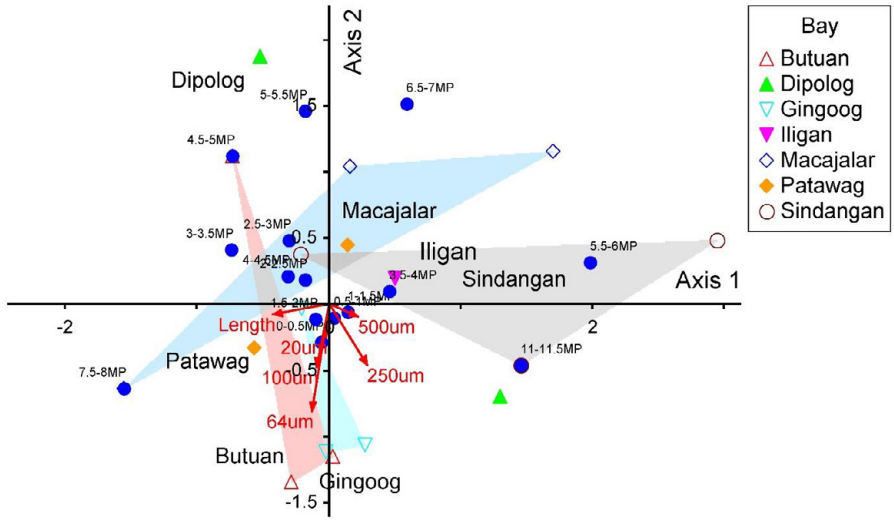

Fig. 5: CCA biplot of bays and densities of microplastic at different size classes with standard length, and size classified food particles of *S. lemuru* as explanatory variables

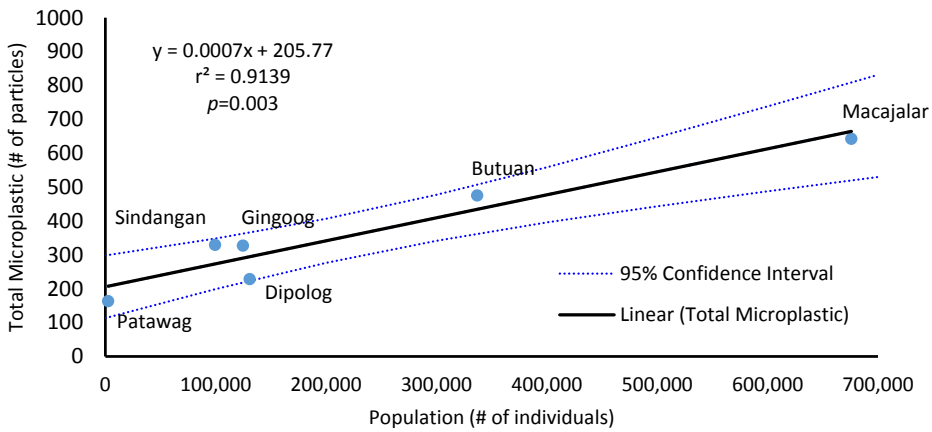

Fig. 6: Relationship of the total ingested microplastics in sardines and the human population in the respective landing sites along Northern Mindanao

methodology and be able to chemically characterize the microplastics using Raman Spectroscopy or FTIR in the future. Most of the local bottled and canned sardine producers maintain a trade secret regarding their manufacturing process. Few others have indicated that they simply cut off the head and tail portions before canning for local and global export market (MEGAGLOBAL, 2018). Philippine culinary tradition includes salting, drying and smoking the whole sardine without removing the entrails. These products are easily accessible at various markets and there is no current regulation that outlines the best and safe practices in processing these sardine goods in the country in spite of the numerous studies had already indicated the adverse human health effects from ingesting food items contaminated with microplastics (Barboza *et al.*, 2018; Karbalaei *et al.*, 2018; Smith *et al.*, 2018; Waring *et al.*, 2018).

CONCLUSION

In conclusion, our study indicated that sardines from the major fishing area in the Philippines were very vulnerable to microplastic pollution. Approximately 85% of the 600 sardines that were collected in various catch landing sites contained 2238

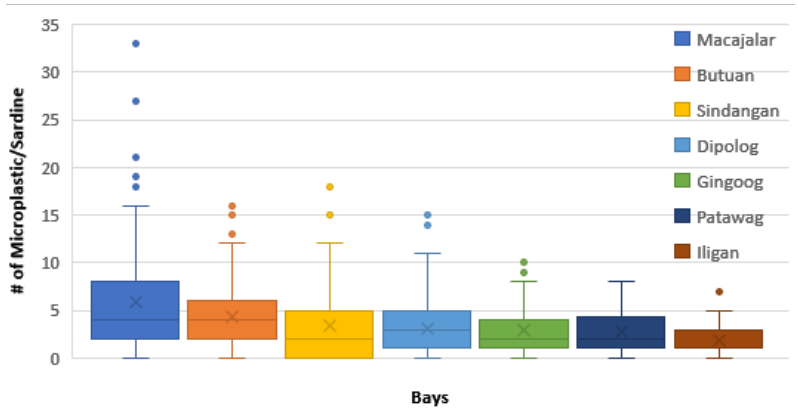

Fig. 7: Summary of ingested microplastics in sardines from the different bays across Northern Mindanao from 2014 to 2016. Data are the sample means (x), median (-), minimum & maximum (whiskers), 25th – 75th percentile (box), and outliers (dots).

microplastics extracted from the stomach of the fish. The average number of microplastics was estimated to contain 3.72 ± 3.97 particles per sardine and the major type of microplastic found was in the form of fiber. Since there is no regulation to minimize microplastic contamination in sardine products, it is likely that the consumers are vulnerable as well to various threats associated to microplastic pollution. Also, the amount of ingested microplastics in sardines may serve as a proxy to indicate levels of anthropogenic pressure exerted on the marine environment by unsustainable practices regarding general use of plastics. It has been found that ingested microplastics in sardines increased with human population in the landing sites. As the demand for plastic use is directly proportional to the size of human population, so as the generated volume of plastic waste that may likely be mismanaged. In the case of the Philippines, mismanaged plastic waste would immediately impact the coastal environment since urban areas are mostly situated along the coasts. Hence, stringent policies to address food safety and proper plastic disposal, if not total prohibition of plastic use, are highly recommended.

AUTHOR CONTRIBUTIONS

J.D. Palermo has performed conceptualization, sample and data analysis, visualization, manuscript preparation. K. Labrador, J. Follante, and A. Agmata contributed in the sample collection and processing, manuscript review and editing. M.J. Pante was responsible in securing the project fund and participated in the manuscript review along with R. Rollon and L. David.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

ABBREVIATIONS

CCA	Canonical correspondence analysis
cm	Centimeter
r^2	Coefficient of determination
^oE	Degrees east
^oN	Degrees north

Ethanol	Ethyl alcohol
Fig.	Fig.
GF/C	Glass microfiber filters
g	Gram
>	Greater or more than
ind	Individual
km	Kilometer
Km^2	Square kilometer
<	Less than
log	Logarithmic
max	Maximum
µm	Micrometer
MP	Microplastic
MS	Microsoft
mg/L	Milligram per liter
mm	Millimeter
MMT	Million metric tons
min	Minimum
#	Number
%	Percent
PPT	Polyethylene terephthalate
PP	Polypropylene
p-value	Probability value
RE	Recovery efficiency
n	Sample size
±	Standard deviation
SL	Standard length
TL	Total Length
Milli-Q	Ultrapure water system

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