

# Microplastic Contamination of Three Commonly Consumed Seafood Species from Taiwan: a Pilot Study

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**Abstract:** Microplastics have already been detected in various human foods, especially seafood. This problem should be especially pertinent to the Taiwanese public because a relatively high proportion of people's diet comes from seafood. We therefore present a pilot study of microplastic contamination of seafood products commonly consumed by Taiwanese people. We examined six batches of three seafood species for the presence of microplastics using FTIR spectroscopy. A total of 107 seafood individuals from three species (hard clam *Meretrix lusoria*, oyster *Crassostrea gigas*, Loligo squid *Loliginidae* spp.) weighing a total of 994 grams yielded a total of 100 microplastic particles consisting of nine different polymer types. 91% of microplastic particles were fragments which likely originated from fragmented plastic debris which was then consumed by the seafood species. The mean number of microplastics/kg was 87.9 microplastics/kg across the three examined species. Given that Taiwanese average about 10 kg of seafood consumption per year, we estimate that a few thousand microplastic particles are annually consumed on average. The methodology of this pilot study can now be used to conduct examinations of more seafood species and samples.

**Keywords:** food security; marine anthropogenic litter; microplastics; plastic pollution, seafood; Taiwan

## 1. Introduction

Aquatic but also terrestrial habitats are increasingly impacted by plastic pollution [1-5]. Global plastic production is increasing exponentially and reached 335 million metric tons in 2016 [6-8]. Of these 335 million tons, approximately 1.4% to 3.8% enter the oceans each year [9].

Once in the environment, plastic objects and fragments have numerous negative impacts. For example, they can damage ships, even causing accidents, cause the injury and death of animals, and damage habitats and ecosystems. When macroplastics degrade into meso-, micro-, and nanoplastics, they can directly enter the food chain or indirectly contaminate it because of the leaching of their potentially harmful ingredients [1, 4, 10-12]. For the purposes of our study, meso-, micro-, and nanoplastics are defined to be the following size categories, respectively: 1 to < 10 mm, 1 to < 1000  $\mu\text{m}$ , and < 1  $\mu\text{m}$ , although different thresholds have been used by various authors [13]. Possible human health impacts of microplastic and nanoplastic pollution are the possibility of internal injury and toxic reactions after ingestion or the indirect contamination of air, food, and water with harmful substances (see Discussion).

Microplastics have already been found in various human foods: beer, drinking water, honey, seafood, sugar, and table salt [14-16]. Ingestion of microplastics has been demonstrated for many marine organisms including zooplankton, bivalves, crustaceans, worms, fish, seabirds, reptiles, and mammals [15, 17]. Between 2% and 40% of fish individuals were found to be contaminated with microplastics [18-20]. Therefore, most humans around the world now ingest various amounts of microplastic (and likely also nanoplastic) particles directly via food, especially when consuming fish and seafood. Because fish and seafood provides more than four billion people with a substantial proportion of their animal protein intake [21], plastic pollution of fish and seafood leads directly to ingestion of microplastic and nanoplastic particles for billions of people.

While this is now an established fact, we nevertheless need to understand much more about the microplastic contamination of seafood, such as: (1) what species are involved; (2) what is the level and variation of contamination; (3) what polymer types are involved; and (4) what is the regional variation of contamination.

Considering the last aspect, we do not have complete geographic coverage yet. Barboza et al. [15] reviewed studies reporting the occurrence of microplastics in shellfish and fish of commercial interest as food, and they listed only one study from China [22], one study which included fish from Japan and Vietnam [23], and none from the Philippines, Korea, or Taiwan. We thus decided to check for microplastics in seafood commonly purchased by Taiwanese people.

The possible problems associated with plastic contamination should be especially pertinent to the Taiwanese public because a relatively high proportion of people's diet is seafood (see Methods). Taiwan's coastline is heavily polluted with macroplastics [24] and microplastics [25, 26], and the waters surrounding Taiwan are amongst the most plastic-polluted in the world [27-30]. While some seafood consumed in Taiwan originates from other parts of the world, there also remains a local fishing fleet which fishes in the waters surrounding Taiwan. To find out if such seafood is contaminated, we conducted a pilot study to examine several seafood products commonly consumed by Taiwanese people for the presence of microplastic particles.

## **2. Materials and Methods**

### *2.1. Selecting seafood species*

Taiwan is a nation state in East Asia consisting of one large island (Taiwan Island) and several much smaller islands and island groups. Its population is approximately 23.5 million people, and it has the 30<sup>th</sup> highest GDP per capita income in the world, with people consuming much local food, but also food imported from all over the world.

In order to determine what species of seafood are commonly consumed by Taiwanese people, we researched publicly available data in September 2017. We found two relevant sources:

1. Chen and Chen [31] provided a list of fishes, shellfish, and cephalopods commonly consumed in Taiwan (Table S1).
2. Taiwan's Fisheries Agency provided estimates of the production quantity of 12 taxa of seafood in Taiwan 2016 (Figure S1), all of which are commonly consumed in Taiwan.

### *2.2. Extracting microplastics from seafood samples*

Based on these two lists of seafood products, we purchased samples of three species, namely the hard clam *Meretrix lusoria* (Veneridae), the oyster *Crassostrea gigas* (Ostreidae), and Loligo squids *Loliginidae* spp. (Table 1) which were randomly chosen from the list of commonly consumed species (Table S1, Figure S1). For each species, we obtained two independent batches (or samples) purchased on different dates and from different sources (Table 1).

**Table 1** Six batches of seafood purchased from markets and supermarkets in Taiwan from September to December 2017.

ID <sup>a</sup>	Common name	Scientific name	Source <sup>b</sup>	No. inds <sup>c</sup>	Mean weight (grams) <sup>d</sup>
1	Hard clam	<i>Meretrix lusoria</i>	Supermarket (Qigu, Tainan)	25	11.62
2	Hard clam	<i>Meretrix lusoria</i>	Fish Market (Budai Township, Chiayi)	25	11.99
3	Oyster	<i>Crassostrea gigas</i>	Wuxing Local Market (Dongshi Township, Chiayi)	27	4.45
4	Oyster	<i>Crassostrea gigas</i>	Fish Market (Budai Township, Chiayi)	24	4.58
5	Loligo squid small	<i>Loliginidae</i> spp.	Wuxing Local Market (Keelung, New Taipei City)	4	14.34
6	Loligo squid medium	<i>Loliginidae</i> spp.	Wuxing Local Market (Keelung, New Taipei City)	2	58.05
Total	3 species	-	4 markets	107	993.78

<sup>a</sup> Each seafood batch was assigned a sample ID (1-6). <sup>b</sup> The source is the type of market where the seafood was purchased (in brackets, we give the original source which the seller indicated when asked about the origin of the seafood). <sup>c</sup> The number of individuals which were digested and examined for microplastics is given. <sup>d</sup> The mean weight of these individuals (whole weight, but after removal from shell for clams and oysters, cf. Methods) is given in grams.

All samples were immediately wrapped in aluminium foil to avoid contamination and were then frozen.

We used each individual animal as a whole sample. Therefore, for squids, we simply placed the whole animal in a 10% potassium hydroxide (KOH) solution. However, for the clams and oysters, we removed all the animal parts from inside the shells and placed all of them into the KOH solution. The rationale was that people in Taiwan consume the entire animal when they cook it for consumption, so using the whole animal most realistically replicates the exposure to microplastics in real-life situations.

We digested animal samples using an already established laboratory protocol using KOH solution. Dehaut et al. [17] conducted a comprehensive comparative test of dissolution methods which overwhelmingly recommended the use a 10% KOH solution because it digested all animal tissues except bones and shells within a sufficiently short duration but did not digest or degrade any of the tested plastic types (except for cellulose acetate) which are most commonly found within seafood. This so-called 1b protocol was therefore adjudged to be superior to five other dissolution protocols.

Nevertheless, we also ran validation trials to further test this method. We used the 1b protocol (summarized in Appendix 1) to run preliminary tests using samples seeded with (1) only microplastic particles and (2) microplastic particles and clean seafood tissue. In each case, we were able to recover the seeded microplastic particles (Appendix S2). We therefore had high confidence in the use of the 1b protocol which we consequently adopted to extract microplastics found within our seafood samples.

The details of our dissolution and extraction protocol are given in Appendix S1. This protocol worked entirely satisfactory for all samples. Briefly, we prepared clean, microplastic-free water using a vacuum pump and filter system, and then used the clean water to produce the 10% KOH solution. After defrosting, we unwrapped the sample and placed it immediately into a new vial which was then filled with the KOH solution in a clean laboratory environment. Vials were placed into 50° C water bath for 24-48 hours until each sample had been completely digested.

We then used the same vacuum pump and filter system to filter each vial's contents separately through a filter paper with a 20-25 µm pore size (Whatman Laboratory Products) which took less than one minute. The filter paper retained any solid particles bigger than its pore size. The filter paper was then immediately placed into a new petri dish, covered with a labelled cover, and sealed with tape. The filter paper in each petri dish was dried at

room temperature for one night. Besides using filtered water and aluminium caps, we further minimized contamination of samples by always wearing a laboratory suit which does not shed fibers [32].

During the laboratory work which we described above, we also always conducted three controls:

1. Blank run: We filled vials with the KOH solution and subjected them to the same procedure as above, but without animal samples in them, and then filtered the solution through a new filter paper.
2. Control water: We filled one flask with 800 ml of filtered water and sucked the water through a new filter paper to check for contamination in the water and/or the filter paper [32].
3. Control air: We poured 800 ml of filtered water into an open wide mouth bowl and placed it directly next to the laboratory equipment so that the water could catch airborne contaminants. At the end of each laboratory run of digesting and filtering one of the seafood products, we then also filtered the water from the bowl through a new filter paper.

### *2.3. Counting and identifying microplastic particles*

We examined each filter paper (including the three controls) using an Olympus BX53 Stereo Microscope (range of magnification 2-10x). We counted the number of potential microplastic particles which we could visibly detect for each filter paper and categorized them into three main groups based on overall shape using the definitions established by Karami et al. [32]: fibers/filaments (thin, straight and often cylindrical particles), fragments (jagged and irregular shaped particles which often have an uneven surface), and pellets (rounded particles). All particles were photographed.

We used FTIR spectroscopy to identify whether a particle was plastic or not and to identify the plastic polymer type. Between January and August 2018, we analyzed potential microplastic particles with the attenuated total reflection Fourier transform infrared (ATR-FTIR) microspectroscopy at the endstation TLS 14A1 of the National Synchrotron Radiation Research Center (NSRRC). This method analyzes the chemical components based on the characteristic IR absorption of functional groups of plastic polymers. The endstation includes a FTIR spectrometer (IFS 66 v/S, Bruker, Ettlingen, Germany)

equipped with an IR microscope (Hypersion 3000, Bruker, Ettlingen, Germany), coupled with a single reflection 20× ATR objective, which is an anvil shaped Ge crystal with an 80  $\mu\text{m}$  in diameter. The FTIR spectra of each plastic particle collected onto a filter paper were acquired with 512 scans and a resolution of 4  $\text{cm}^{-1}$  in the spectral range of 4000 – 400  $\text{cm}^{-1}$  by using the endstation of the ATR-FTIR microspectroscopy. We focused the infrared radiation onto the Ge crystal of the ATR objective and contacted each plastic particle for acquiring FTIR spectrum of individual plastic particle. To ensure an FTIR spectrum free of spectral interference from water and carbon dioxide in the atmosphere, the optical path of the endstation of the ATR-FTIR microspectroscopy was continuously purged with dry nitrogen evaporated from the LN Dewar (XL-100, TAYLOR- WHARTON, Theodore, AL, USA) during each FTIR data acquisition. This equipment has been used for other studies of microplastic pollution before [25, 26].

To determine the polymer type of each particle, we compared each particle's spectrum with those stored in a FTIR polymer spectrum library using the OMNIC<sup>TM</sup> software (OMNIC 9.2, 2012; ThermoFisher Scientific Inc., Waltham, MA, USA) which automatically matches a measured spectrum to a list of reference spectra from many different materials.

#### *2.4. Measuring the size of microplastic particles*

While we determined the polymer types of each particle, we measured the sizes of only a subsample of the particles because of time constraints. Due to time constraints, a random sample of 65 out of the 100 particles was chosen, and then each particle's size was measured with a scale build into the endstation's microscope (Table S4). To measure the size of each particle, the scale built into the microscope was adjusted to measure the maximum dimension of the particle.

The minimum size which we could detect and measure with this setup was 10  $\mu\text{m}$ , so this study detected only microplastic particles in the size range of 10 to < 1000  $\mu\text{m}$  (cf. [13]).

### **3. Results**



To obtain our results, we purchased, digested and examined six batches of three seafood species purchased from two markets and one supermarket (Table 1). A total of 107 seafood individuals weighing a total of 994 grams were examined among which we detected a total of 100 microplastic particles consisting of nine different polymer types (Tables 2, S4). We used these results to calculate estimated microplastic particles per kg of fresh seafood tissue and derived estimates of 213.4 and 20.0 microplastics/kg for hard clams (mean = 116.7), 124.8 and 91.0 microplastics/kg for oysters (mean = 107.9), and 34.9 and 43.1 microplastics/kg for loligo squids (mean = 39.0). The mean number of microplastics/kg across the three species was  $((116.7 + 107.9 + 39.0) \text{ particles/kg})/3 = 87.9 \text{ microplastics/kg}$  of fresh seafood.

**Table 2** Number and polymer type of microplastic particles detected in six batches of seafood.

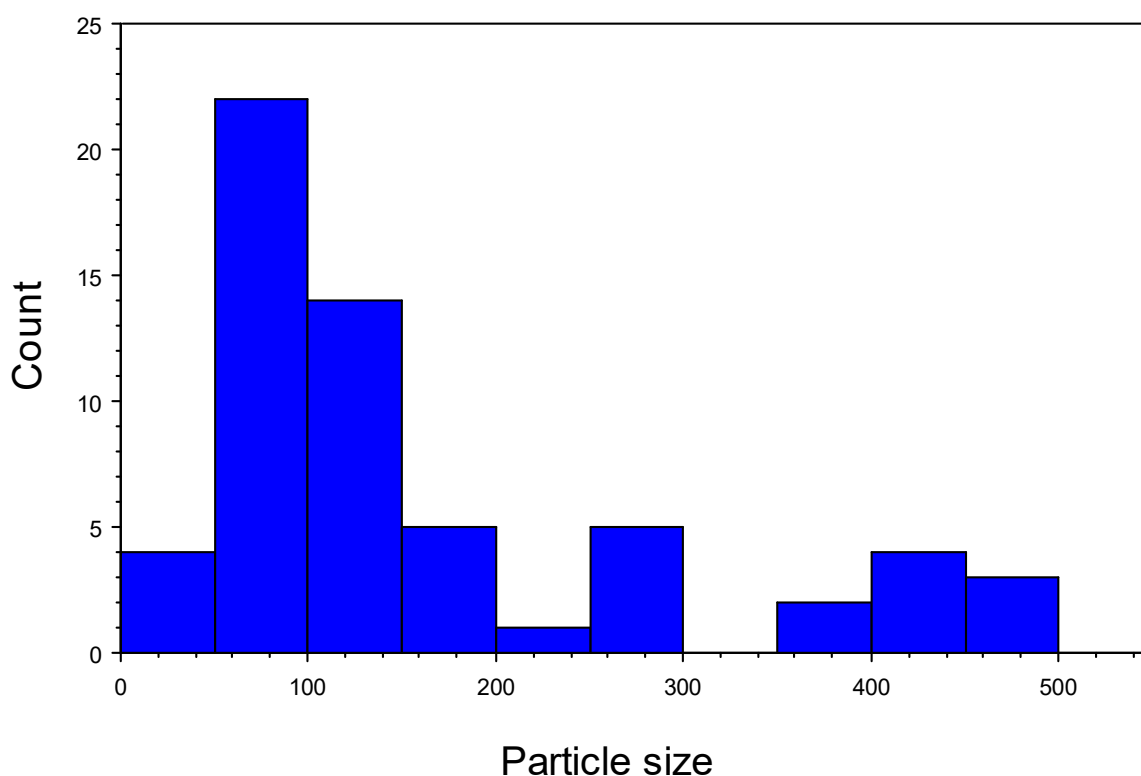
ID	Common name	Total no. <sup>a</sup>	No. per kg <sup>b</sup>	Polymer Types of Plastic Found (Amount) <sup>c</sup>
1	Hard clam	62	213.4	PP (50), PET (5), PEA (4), PMMA (2), PA (1)
2	Hard clam	6	20.0	PP+PE-PP (3), PA (1), PE-LD (1), PET (1)
3	Oyster	15	124.8	PA (5), PET (3), PEA (2), PE-PP (2), PP (2), PE (1)
4	Oyster	10	91.0	PE-PP (7), PE-LD (2), PET (1)
5	Loligo squid small	2	34.9	PE-PP (1), PET (1)
6	Loligo squid medium	5	43.1	PE-PP (2), PE (1), PEA (1), PP (1)
Total	mean across 3 species	100	87.9 <sup>c</sup>	PP (53), PE-PP (12), PET (11), PA (7), PEA (7), PP+PE-PP (3), PE-LD (3), PE (2), PMMA (2)

<sup>a</sup> Total number of microplastics and <sup>b</sup> number of microplastics per kg of fresh seafood tissue detected within the six batches of seafood (Table 1). <sup>c</sup> The types of plastic polymers detected are given (for abbreviations, see Table S3); brackets behind each abbreviation give the number of microplastic particles of that particular polymer type detected in each batch. The raw data are presented in Table S4. <sup>c</sup> Calculation is presented in the Results.

By far the most common polymer type was polypropylene with 53 out of the total of 100 particles (53%), followed by poly(ethylene:propylene:diene) (12 particles) and polyethylene terephthalate (11 particles); the remaining six polymer types had seven particles or less (Table 2). Examples of typical spectra for each of the nine identified polymer types are shown in Figure S2. The two most common polymer types were present in all three species, but none of the others were.

The microplastic particles had the following shapes: 8 were fibers (3 grey, 2 black, 1 blue, 1 transparent, 1 yellow), 91 were fragments (33 blue, 31 green, 9 white, 7 black, 5 transparent, 3 grey, 2 brown, 1 dark blue), and one was a transparent pellet. The sizes of the particles were  $190.5 \pm 192.4 \mu\text{m}$  (mean  $\pm$  St. Dev.;  $n = 65$ ; range 20-800  $\mu\text{m}$ ) with the size distribution being right-handed (Figure 1).

**Figure 1.** Histogram of the size distribution (in  $\mu\text{m}$ ) of the microplastic particles.



No microplastic particles were found in our blank, ‘control air’ and ‘control water’ samples. These results strongly suggest that environmental contamination with microplastics did not occur during our laboratory work, but that the microplastics identified by us originated from the seafood products themselves.

#### 4. Discussion

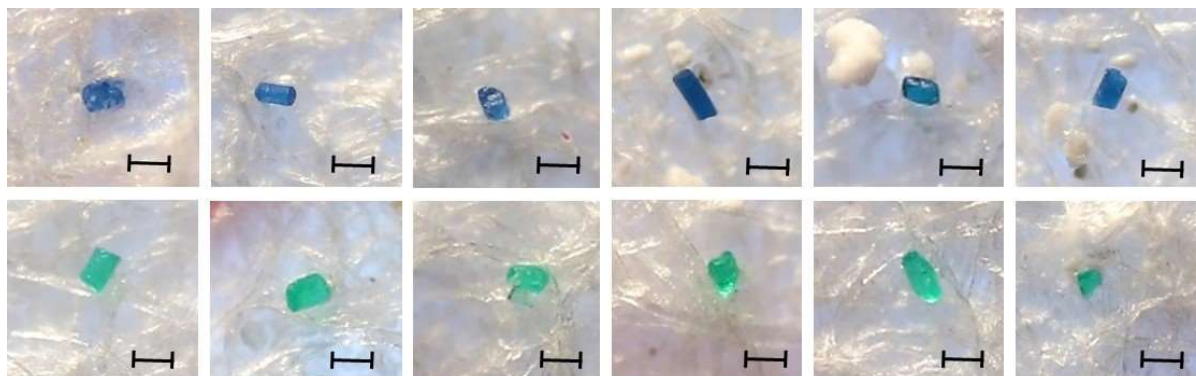
We unequivocally demonstrated microplastic contamination of three species of seafood commonly consumed in Taiwan. The identification of plastic polymers by FTIR spectroscopy is one of the most widely accepted methods to confirm the presence of microplastic contamination; therefore, our results cannot be influenced by possible problems with visual identification [14].

The fact that 91% of particles were fragments, 8% were fibers, and 1% was pellets somewhat reflects the contamination of Taiwan’s environment: on sandy beaches, 88.6% of particles were fragments, 0.2% were fibers, and 11.2% were pellets [25] whereby the number of fibers is likely an underestimate because of the sampling method (A. Kunz, pers. commun.). Therefore, the proportions in seafood are roughly similar to the proportions found on sandy beaches (despite the fact that most microplastics recovered from the beaches are substantially larger).

The fact that these three types of microplastic shapes had such varied colours suggests that their sources and pathways into seafood were probably also very varied. We assume that most particles originated from the fragmentation of intact plastic debris which then entered the species when the species fed, as such fragments dominate the microplastic pollution detected around Taiwan [25, 26]. However, some microplastics may also originate from the manufacturing and packaging processes [14, 16, 32, 33]. In the hard clams, we found 26 blue and 24 green polypropylene fragments (Figure 2), which is 94.3% of all the polypropylene fragments which we identified (the remaining one and two white polypropylene fragments were found in a squid and oysters, respectively). Many of these fragments had a regular shape, such as cylinders or rectangles, and looked somewhat similar (Figure 2). It is possible that all of these polypropylene fragments originated from some unknown source during the manufacturing or packaging process, as we find it unlikely that hard clams would ingest such similar particles during the growth stage.

However, without much more extensive detective work, it remains impossible to establish the exact pathways of how microplastics end up in seafood. Nevertheless, the general pathway from ocean pollution to the inside of fishes and seafood is an obvious and well-established pathway (e.g., [34-36]).

**Figure 2.** Six examples each of the blue and green polypropylene fragments found in hard clams. The scale bar indicates 100  $\mu\text{m}$ .



We detected nine different polymer types, with the most common by far being polypropylene, polyethylene terephthalate, and polyethylene. This result is to be expected because these three types are among the most widely used for packaging and other common uses, and they were the three most abundant types in a global survey of microplastics in table salts [14], while polyethylene and polypropylene were the two most abundant types in a global survey of microplastics in the marine environment [37], in a study of Pacific fishes [38] as well as in two studies of microplastics on Taiwan's beaches [25, 26]. Two of the three remaining types found in our study (namely PA and PMMA) were also detected in table salts [14] and the marine environment [37]. Furthermore, microplastics found in other studies of wild fishes and seafood also included polyethylene [22, 34, 35], polyethylene terephthalate [22, 39], and polypropylene [35], with all three being among the most reported types in a global review [40]. Therefore, the polymer types which mainly contaminated our seafood samples are typical representatives of the global plastic pollution problem.

The mean and range of our particles' sizes were also well within the reported range of microplastics in other foods; e.g., Lee et al. [14] reported a range size of 4-7000  $\mu\text{m}$  in their global review.

The microplastic concentrations of hard clams (116.7 microplastics/kg) and oysters (107.9 microplastics/kg) were similar and their ranges overlapped while the concentrations for squids (39.0 microplastics/kg) were clearly lower (Table 2). One possible explanation for this result is that clams and oysters are filter feeders and thus have similar feeding mechanisms that are different from the feeding mechanism of the squids which are carnivorous predators of various small marine animals. However, controlled laboratory experiments would be needed to establish whether feeding mechanisms are indeed related to rates of microplastic ingestion in these three species.

De Witte et al. [36] found between 260 to 510 microplastics/kg in the blue mussel *Mytilus edulis*, while, for the same species, Van Cauwenberghe et al. [34] reported 200 microplastics/kg and Li et al. [39] detected between 900 to 4600 microplastics/kg. Li et al. [22] examined nine species of commercial bivalves and found between 210 to 1050 microplastics/kg. For the oyster *Crassostrea gigas*, Van Cauwenberghe and Janssen [41] reported 470 microplastics/kg. These levels of contamination are all somewhat higher than ours, but only by a factor of two to four at the lower end. Such differences can be due to real differences in the level of contamination or to different laboratory procedures [14].

The yearly mean consumption of shellfish in Taiwan was 197 g/week for men and 200 g/week for women in the 1990s (Table 2 in Chen and Chen [31]). Taking the mean of 198.5 g/week, this yields an annual consumption of 10.35 kg/year. Multiplying this estimate with our mean of 87.9 microplastics/kg yields an annual intake of about 909.8 microplastics/year. This estimate of shellfish consumption is based on the Nutrition and Health Surveys in Taiwan (NAHSIT) for the period 1993-1996 [42]. A subsequent NAHSIT for the period 2005-2008 found an approximately 35% increase in the consumption of 'fish, seafood and products' [43]. If we assume that the increase was actually 35% for seafood alone, then the annual consumption would have risen to 14.0 kg/year and the annual intake to 1230.6 microplastics/year. While the actual numbers may be somewhat different, the general magnitude (~ a few thousand microplastic particles) is probably a good preliminary working estimate. In comparison, our numbers are relatively

low; e.g., it was estimated that European seafood consumers could be exposed to up to 11,000 microplastic particles per years, and Chinese seafood consumers even to hundreds of thousands of microplastic particles per year [21]. Naturally, further studies need to be conducted, including more species (see Table S1, Figure S1) and more sources from across the country, and a more current estimate of seafood consumption would also be desirable.

To be best of our knowledge, this is the first study published in the scientific literature which demonstrates the presence of microplastics in seafood commonly consumed by Taiwanese people. However, since we began our work in 2017, we have become aware of two other relevant research projects. The first one is a Chinese-language report conducted by Taiwan's Environmental Protection Administration (EPA) [44] which found microplastics in clams, mussels, scallops, and wild and farmed oysters. The second study is an unpublished thesis supervised by National Donghwa University marine biology professor T.-h. Chen; the study found that about 95% of coral fishes and nearly all of 21 turtles had microplastics in their digestive tracts [45] which substantiates many earlier findings of microplastic contamination of ocean-living species (see References above).

Hence, three studies have found substantial numbers of microplastics in various seafood and fish samples from Taiwan and mutually substantiate each other's main findings, including the possible effects on human health (see below). The fact that microplastics are found in seafood worldwide has thus also been verified for Taiwanese seafood. Other recent studies also verified the presence of microplastics in table salts purchased in Taiwan [14, 16, 46].

The possible human health effects of the consumption of microplastics and nanoplastics have been addressed an increasing number of publications [11, 15, 21, 47-56]. Three possible impacts have been postulated: direct ingestion of microplastics and nanoplastics and the possible internal injury; the indirect contamination of air, food, and beverages, including water; and the possibility of microplastics serving as pathogen vectors. Hwang et al. [57] recently demonstrated the potential toxicity of polystyrene microplastic particles at the cellular level. Whether these possible impacts result in actual significant health risks is still largely unknown. GESAMP [56] concluded that 'the potential ecological and human health risks of microplastics are relatively new areas of research, and there is currently a large degree of uncertainty surrounding this issue,' which is the conclusion largely shared

by Miller et al. [55]. Similarly, the EFSA Panel on Contaminants in the Food Chain [54] suggested that microplastics in seafood probably have a small effect on people's overall exposure to additives or contaminants.

Environmental plastic pollution can be alleviated to some extent by cleanup activities [24, 58] but much better are source-reduction policies [27, 59, 60]. Therefore, the recently announced future ban of single-use plastic products by the Taiwanese government should be welcomed [27, 61, 62].

In conclusion, we found that all seafood products from Taiwanese markets contained microplastic particles, although in highly varying amounts (by one order of magnitude). As pointed out above, there is no consensus yet to clarify whether significant health risks exist and what quantity of ingestion of microplastic particles might be considered harmful or not. However, exponentially increasing amounts of plastic are entering into various habitats, whereby this plastic pollution clearly impacts ocean ecosystems the most. Consequently, seafood coming from the ocean are contaminated with microplastics and nanoplastics. Until we know more about the potential health effects, this fact remains worrying, especially given that this impact will increase in the coming decades. In light of our findings, we recommend that the Taiwanese government and its scientific research community continue to monitor and study macroplastic, microplastic, and nanoplastic contamination, and their possible effects on human and animal health.

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**Conflicts of Interest:** The authors declare that there is no conflict of interests regarding the publication of this paper.

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