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Short Communication

Presence of Microplastics in Four Types of Shellfish Purchased at Fish Markets in Okayama City, Japan

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The worldwide microplastic pollution in our environment is a matter of great concern. Harmful effects of plastics have been reported in various types of organisms including murine animals. We examined the presence of microplastics in four types of shellfish purchased from fish markets in Okayama, Japan and served to the public: short-neck clam (Ruditapes philippinarum, *asari* in Japanese), hard-shell clam (Meretrix lusoria, *hamaguri*), brackishwater clam (Cyrenidae, *shijimi*), and oyster (Crassostrea gigas, *kaki*). Our analyses demonstrated that approx. 3 pieces of microplastics were present per single shellfish, based on the division of the total number of pieces of microplastic obtained from all 4 types of shellfish by the total number of shellfish examined. Since health problems in humans due to microplastics have not yet been confirmed, further examinations of the effects of ingested microplastics are needed.

Key words: microplastics, shellfish, Japan, health effect, pollution

Approximately 448 million tons of plastic were produced worldwide in 2015, and about half of that was disposed of in the environment. The disposed plastics are broken down into microplastics (defined as < 5-mm-long fragments of plastic) by natural forces such as sunlight, waves, wind, and heat [1,2]. Song *et al.* reported a large accumulation of micro-sized synthetic polymer particles in the sea [3]. Microplastics have thus become a serious environmental and public health issue [2], and many governments and environmental entities in various countries are working to resolve the problems presented by microplastics.

Microplastics were found in the digestive tracts of Japanese anchovies sampled from Tokyo Bay [1], and many other cases of microplastic ingestion by fish are cited in that report. Jeong *et al.* observed reduced growth and fecundity in marine copepods that engulfed microplastics, possibly due to a physical disturbance of the digestive system [4]. The presence of ingested microplastics in the mussels was described by Browne *et al.* [5], and Napper *et al.* reported that washing clothes could be a source of thread-type microplastics [6]. We conducted the present study to determine whether or not four types of shellfish that are commonly consumed by Japanese are contaminated with microplastics.

Materials and Methods

Sampling. The shellfish samples were obtained at fish markets in the city of Okayama, Japan. The production district of each shellfish is shown in Table 1. Each sample (the number of each shellfish shown in

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Table 1) was incubated and shaken in 400 ml of 1%HCl (Wako, Hiroshima, Japan) solution with 1% pepsin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 4 h to digest the protein. The digested samples were then passed through a 100-μm pore-size mesh fabric sheet (Taiyo, Osaka, Japan). The remaining residue on the sheet was treated with 5 ml of 10 N NaOH (Wako) at room temperature for 24 h to break down organic materials. After this processing, the samples were again passed through the same mesh fabric sheet and the residues were washed by tap water, followed by drying.

The samples were then examined under a light microscope, and the number of microplastics was counted and evaluated. Photomicrographs of the samples were taken using a BZ-X700 microscope (Keyence, Osaka, Japan).

Identification of plastics. The microplastics obtained from the shellfish were analyzed by an infrared spectrometer (Compact FTIR 5500a, Diamond ATR, Agilent Technology, Anta Clara, CA, USA). The identification of the microplastics was carried out by comparison with standard controls including HDPE (high-density polyethylene), LDPE (low-density polyethylene), PET (polyethylene telephtalate), and PS (polystyrene).

Results and Discussion

When we examined the prepared samples by light microscopy, we detected many pieces of thread as well as microplastics. Figure 1 provides a photomicrograph of the short-neck clam (*asari*) sample. The pieces of thread may be derived mostly from clothes made of plastic materials in the process of washing [6]. We did not analyze the types of threads, but they may be from polyester, polyester-cotton blend, and acrylic fabrics [6].

The peaks of the microplastic obtained from short-neck clam using a Fourier transformation infrared spectrometer (FTIS) were coincident with polyethylene standard peaks (Fig. 2). We thus determined that the microplastic from the short-neck clam was polyethylene.

We carried out the experiment in order to detect microplastics in the shellfish twice. The first experiment was done in January 2019, and the second was performed in November 2020 to compare the findings with the results of the first experiment. Table 1 provides the number of microplastics and pieces of threads in each shellfish counted under a microscope. Although not all of the microplastics fragments identified under the light microscope could be examined by FTIS due to their small size and fragile state, they were surely

Table 1 The amounts of microplastics and threads in four types of shellfish

Samples*	Experiment 1 (Jan, 2019)**			Experiment 2 (Nov, 2020)**		
	Sample district	Numbers /Shellfish Plastics Threads	Numbers /100 g Plastics Threads	Sample district	Numbers /Shellfish Plastics Threads	Numbers /100 g Plastics Threads
Short-neck clam (*Ruditapes philippinarum*)	Kumamoto, Japan	2.4 1.8	140 105	Kumamoto, Japan	3.4 3	252 222
Hard-shell clam (*Meretrix lusoria*)	China	2.6 2.5	96 93	China	8.7 4.7	613 331
Brackishwater clam (*Cyrenidae*)	Aichi, Japan	1.6 0.5	416 130	Okayama, Japan	0.4 0.9	121 273
Oyster (*Crassostrea gigas*)	Okayama, Japan	2.8 2.4	16 14	Okayama, Japan	4.6 7.8	63 103

*Short-neck clam is called as *asari* in Japanese, Hard-shell clam is *hamaguri*, brackishwater clam is *shijimi*, oyster is *kaki*.
**For both experiments, 10 asaris, 10 Hamaguris, 20 Shijimis, and 5 kakis were used.
For experiment 1, the asari, hamaguri, shijimi and kaki weighed 17.2 g, 27.0 g, 7.7 g, and 85.3 g, and for experiment 2, they weighed 13.5 g, 14.2 g, 6.6 g, and 37.9 g, respectively. The number of microplastics and pieces of thread in each shellfish were counted under a light microscope.

microplastics, because the samples were fully digested with a strong alkaline solution to decompose the organic compounds. Taking the results of experiment 1 and 2 together, we calculated that approx. 3 pieces of microplastic were present in each single shellfish. Although the numbers of microplastics and threads in

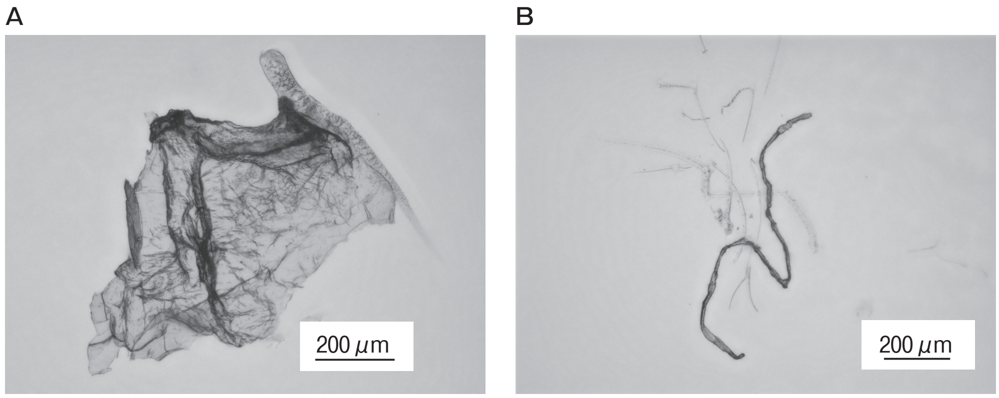

Fig. 1 Photo of a short-neck clam (*asari*) sample. Photomicrographs of the sample were taken using a BZ-X700 microscope. **A**, Microplastics; **B**, Thread; Scale bar, 200 μm.

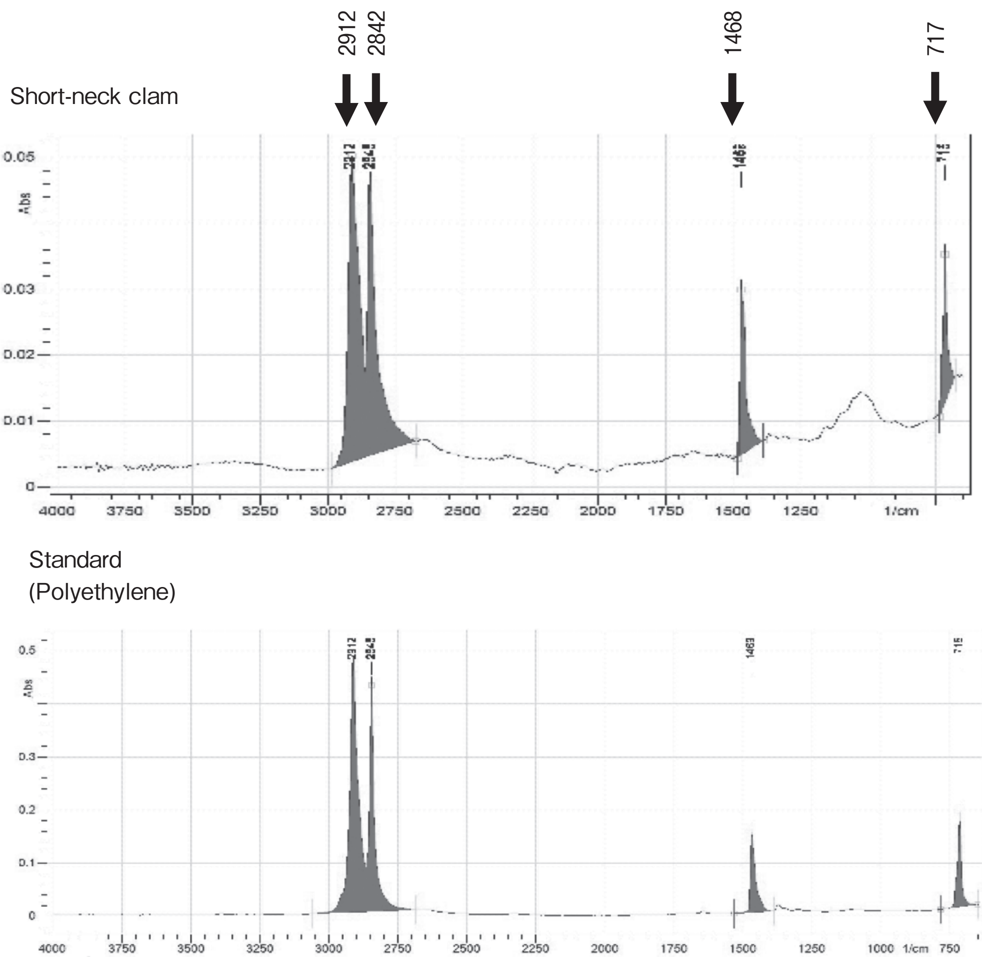

Fig. 2 Analysis of the microplastic shown in Fig. 1 **(A)** and the standard control of low-density polyethylene **(B)** by the FTIS.

each shellfish differed somewhat between the two experiments, the differences may be due to the seasons and/or the districts where the shellfish were obtained.

The precise location of the microplastics materials in each shellfish (*e.g.*, the digestive tract or surface folds of the body) could not be determined due to difficulty with the dissection step. However, the location of the microplastics is not so critical since the shellfish are eaten whole by boiling, baking, or frying. Intriguingly, Scwable *et al.* detected various microplastics in human stool [7]. Since microplastics are ubiquitous in natural environments, they are certainly in our food chain.

Microplastics themselves may cause physical disturbance in the digestive system of consumers, and further degraded, nano-level materials can enter cells through endocytosis, resulting in the damage of cell functions. Moreover, substances leached from the ingested microplastics may be more dangerous to cells than are plastics.

In our previous investigation, alcohol extracts of a commercially available polyvinylidene chloride (PVDC) wrap were cytotoxic to cultured human liver cells and mouse primary cultured liver cells [8]. Then, we determined that the toxicity is due to the plasticizing agents in PVDC (*i.e.*, epoxidized soybean oil and epoxidized linseed oil) were toxic to the cultured liver cells but the plastic itself was not toxic to them [8]. Lithner *et al.* reported similar results in which the leachates from many plastic products were toxic and caused the immobility of the small crustacean Daphnia magna [9]. Oehlmann *et al.* reviewed the biological impacts of plasticizers on wildlife [10].

Considering these facts, the effects of human's ingestion of microplastics with their food should be carefully monitored. Last but not least, a reduction in the use of plastic products in our daily life is the best way to prevent microplastic pollution in our environment.

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