ASSESSMENT OF MICROPLASTICS CONTAMINATION IN COMMERICIAL CLAMS IN THE COASTAL ZONE OF VIETNAM

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Abstract. Microplastics (MPs) present in aquaculture farms represent a big issue globally due to their effect on human health. Both vertebrates and invertebrates have been found to ingest microplastics in coastal areas. However, in Vietnam, the impact of microplastics on aquaculture farms is not yet well understood because there has been no research on the presence of microplastics in local clam species even though they are a common food safety concern. This study aimed to understand the ingestion of microplastic contamination in local clam samples of two species (*Meretrix lyrata and Tapes dorsatus)* from household aquaculture farms. In this study, a total of 182 particles consisting of fibers (67%) was separated and the EVA (18%) was identified. Furthermore, the number of MPs was correlated with the clam's weight and soft tissues during the sample taking. In terms of the distribution of the MPs, efficient management of environmental pollution should be considered in the coastal areas as it is so crucial for human seafood consumption in the long-term future.
Keyword: *aquaculture, coastal areas, farming, local clam, species*

Introduction

In the marine environment, microplastics of all types are rapidly proliferating. At the same time, society makes ever-increasing demands on the aquatic environment, such that the environmental resource management practices of the past need to be reconsidered (Narmatha Sathish et al., 2020a., Strady et al., 2020., Groesbeck et al., 2014). The effect of the persistent plastic contamination on the environment cannot simply be ignored (Hall et al., 2015). A growing body of research shows plastic is ubiquitous globally (McNeish et al., 2018) and plastic contamination in the marine environment has gained intensive consideration (Wu et al., 2020). There is no doubt that microplastic is potentially harmful to all marine life forms that ingest it. Many researches into microplastic ingestion run short-term investigations in areas where plastic pollution has exponentially grown (Azad et al., 2018; Beer et al., 2018; Pradit et al., 2020, 2021). Furthermore, during the MPs progress of marine species, these particles can enter the cycling of the food web (Bergmann, 2009). Furthermore, marine plastic contamination also affects the society, economy (Wu et al., 2020), ecology, and biology (McNeish et al., 2018; Botterell et al.,

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2019; Wu et al., 2020). Marine organisms like the clam species are greatly exposed to microplastic pollution even though they have constituted an important part of the everyday seafood produce sold in local and international markets from ancient times, today the clam species are contaminated because of their exposure to microplastic pollution from phytoplankton that have attached to minute particles of plastic found throughout the world's oceans. It may affect on human health when they eat polluted clams (Botterell et al., 2019).

Microplastics are widely scattered in all marine environments (Rillig et al., 2017; Wu et al., 2020). The size of microplastic particles is from 5 mm to 0.1 μm (Lippiatt et al., 2013; Rillig et al., 2017). Furthermore, plastics of different sizes enter the marine environment as particles up to 5 mm in length (Arthur et al., 2009) with larger pieces broken up through physical and mechanical fragmentation (Li et al., 2020). Microplastic debris proliferates, migrates, and accumulates in natural habitats and is scattered on the ocean surface (Ivar and Costa, 2014; McNeish et al., 2018). For instance, fish can transfer microplastic particles to greater trophic levels and humans from zooplankton and phytoplankton (Wu et al., 2020). Even, some inedible parts of fish used in aquaculture or pig raising could be used as feed material for other animals. As a result, the microplastic particles could be continually spread through food as a health hazard (Botterell et al., 2019).

Carpenter et al. (1972) predicted that microplastic contamination would influence several forms of ecosystems and organisms. The microplastic particles are easily broken down by many marine organisms through the ingestion process (Botterell et al., 2019). Therefore, there is a speedily developing awareness of general marine and plastic pollution (Beer et al., 2018). There has been some research on microplastic contamination in the digestive tracts of commercial fish (McNeish et al., 2018; Wu et al., 2020), earthworms in soil (Rillig et al., 2017), Pacific Oyster Larvae (Cole and Galloway, 2015), Zooplankton in the Northeast Pacific Ocean (Desforges et al., 2015), seafood (Chen et al., 2020), and the mussel, Mytilus edulis (Browne et al., 2008). Recently, some researchers have also expressed concern about the clam gardens on the northwest coast (Groesbeck et al., 2014). However, up to now, there have been few studies on microplastics in Vietnam. Furthermore, as yet, none of the studies have focussed on microplastic contamination in clams (*Meretrix lyrata and Tapes dorsatus*) anywhere.

There are two clam species: the *Meretrix lyrata* species in Nam Dinh province and the *Tapes dorsatus* species in Quang Ninh province on intertidal beaches along the coastal waters of North Vietnam (Baechler et al., 2020). Vietnamese government has issued many policies to encourage clam aquaculture based on the situation of coastal zones.

The clam species is not only valued as a chief traditional food value and popular seafood (Li et al., 2015), but is also valued as an essential form of income for the coastal communities. Historically, these species are the main traditional food and an essential source of income for aquacultural farming members (Crosman et al., 2019).

To better understand the pollution in farming (clam species), the objective of this study is to perceive the occurrence of microplastics in clam species from coastal commercial aquaculture. Furthermore, the relationships between different kinds of plastic particles in the tissues of and biological features of clam will be examined.

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Materials and methods

Study area

The study was implemented on one oceanic region in Ban Sen commune (latitude 20°58' N and longitude 107°27''E), Van Don district, located on the edge of Quang Ninh province in the northeast of Vietnam, near the world heritage area of Ha Long Bay (*Fig. 1*). Ban Sen commune has access to diverse aquatic life such as a variety of pelagic fish and clams. It also has other aquacultural resources. Recently, aquaculture has mainly focused on clam production as the main source of income for this area. There are around 150 households with 2.600.000 clam cages. In the last year, the production of aquatic products (clams and oysters) in the commune was estimated at 2.250 tons.

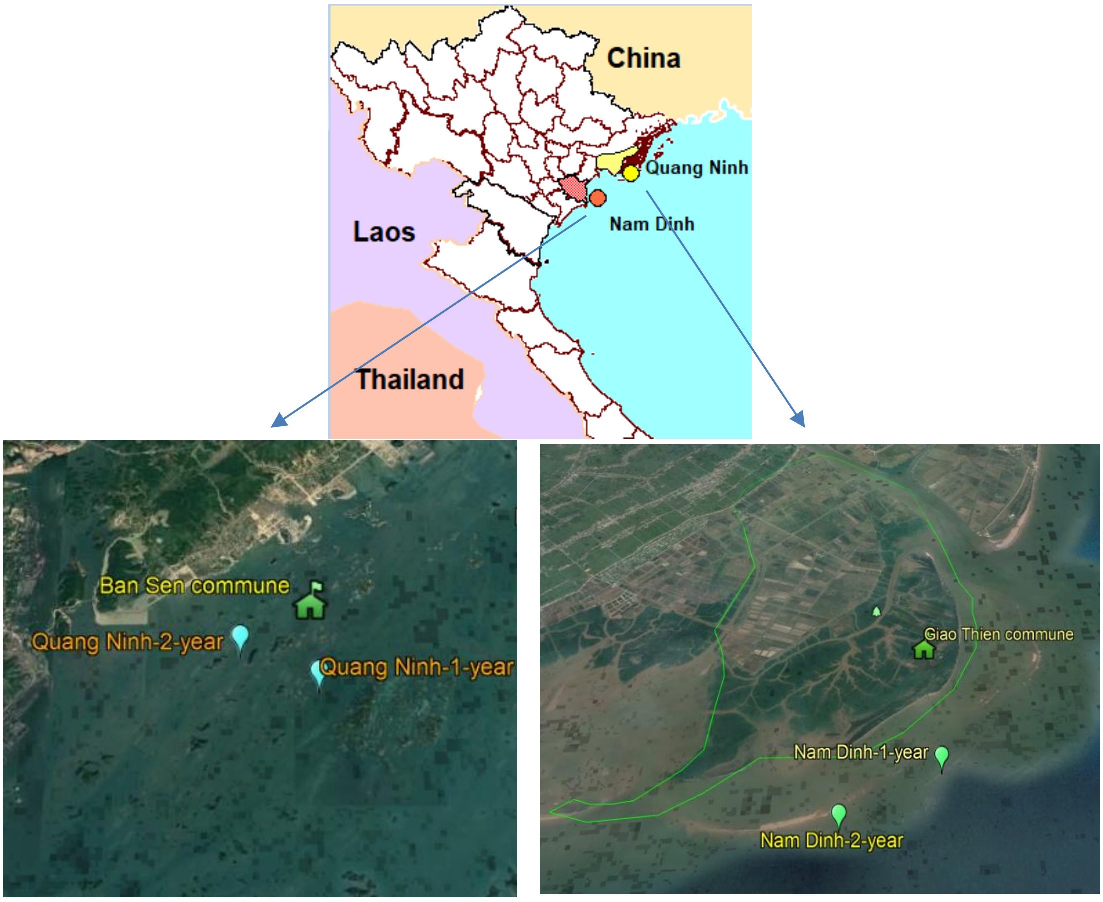

Figure 1. *Location map of studied areas*

Giao Thien commune, that is close to Xuan Thuy national park, is one of the nine Ramsars of Vietnam located in the ocean areas (latitude 20°16' N and longitude 106°32''E) (*Fig. 1*). Local people have aquaculture farms along the coast. There are around 350 households (of a total 8.250 local households) that have been working in aquacultural production, producing 1.360 tons of seafood from 320 ha, which provides the main income.

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Sample collection

Clam species are sampled from the aquacultural farms in the coastal waters of two locations, Ban Sen and Giao Thuy communes, in Quang Ninh (QN) and Nam Dinh (ND) provinces respectively. Clam samples were randomly collected from locations such as: 1-year (latitude 20°02′N; longitude 107°28′E) and 2-year (latitude 21°02′N; longitude 107°28′E) in Ban Sen; 1-year (latitude 20°12′N; longitude 106°34′E) and 2-year in Giao Thuy commune during August 2020 and February 2021 from an average sea depth of ~12 m (Hall et al., 2015). The clams were brought up by using local skin-divers from the commune. Information on clam aquaculture from farming households in the location were noted. There were sampling clam group (2 sites*10 sample groups = 20; total 20*10 = 200 sample clams) across all sites of Ban Sen commmune *(Meretrix lyrata)* and Giao Thuy commune *(Tapes dorsatus)*. At each of the exposed intertidal sandy beaches clam specimens were transferred to the laboratory in an icebox (Li et al., 2018).

Sampling condition control

The clam samples were carefully cleaned, iced, and transported back to the Faculty of Environment, University of Science, Vietnam National University-Ho Chi Minh and stored under laboratory conditions from 2-4 weeks for analysing (Hue et al., 2018a). After a thorough washing with clean water, the clams were weighed and their lengths were measured, and they were then left in the refrigerator (*Fig. 2*). Each clam was measured in terms of age, and the location where it was found (Fig. 3) was recorded. The collected samples were held in a styrofoam case with sufficient ice and transported to the laboratory where they were quickly frozen and maintained at -19°C in the refrigerator (Cole and Galloway, 2015) to keep the stable sampled clams from decomposing until they were further needed (Azad et al., 2018) (*Fig. 2*). The tissues of 10 samples were randomly packed together as one sample (Narmatha Sathish et al., 2020) that were put into pouches made of aluminium foil (Li et al., 2018). The sampled clams from the refrigerator were slowly defrosted and thoroughly rinsed with clean water to remove sand and impurities from their bodies .

Figure 2. *Samples in electric refrigerator*

Figure 3. *Measurement of samples*

Chemical treatment

Clam samples were allowed to defrost for 2 h at normal temperature. The soft tissues of the clams on the cutting board were carefully separated from the shells with a knife, and the wet weight of each specimen was recorded (Narmatha Sathish et al., 2020)

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(*Fig. 4*). In brief, the soft tissues were washed with distilled water to separate the natural debris on the outside prior to working with the clams (e.g., sand, algae, and coral) (Li et al., 2018; Oceanography et al., 2018). The soft tissue was cut into 6-8 small pieces and put into Onelab 100 ml glass tubes (1 ml Ex 20^0C +-1 ml) for determining volume. The tissue was decomposed with Potassium hydroxide (KOH 10%; 3 x tissue volume) in an ED115 convection oven at 56^0C for a period of 48 h (12 h shaking-time) until a clear solution formed (Narmatha Sathish et al., 2020).

Floatation and filtration

After the tissue was decomposed in KOH in the electric oven and was washed in distilled water, all the rotten soft tissues were filtered through a 53 μm sieve and put into a WERTLAB GERMANY glass tube with 100 mL of a mixture of NaCl (1.2 g/ml) and $ZnCl_2$ (1.8 g/ml) (d=1.5 g/cm^3) for 24 hours to float the MPs. The filter paper (Glass microfiber filters) was weighed (*Fig. 4*). The floating residue was filtered through the filter paper with distilled water, using a Rocker 300 vacuum pump with the following settings: 105 m/bar, maximum flow rate, 20-23 liters/min, Rotation speed 1750 RPM, horsepower 1/8 HP, noise level 50 dB, and a voltage of 110 V/60 Hz and 220 V/50 Hz, was used to dry the filter paper at 56^0C (*Fig. 5*).

Figure 4. *Weight of soft clams*

Figure 5. *The soft clams in the ED115 autonomic oven*

Extraction of microplastic

The particles in the glass microfiber filter papers were examined using a USB Digital Microscope with 300x magnification and a 5M pixel image sensor connected to the computer. A visual assessment was made to identify the types of microplastics according to the physical characteristics of the particles (Li et al., 2015). The MP particles (size >0.25 mm) were removed to the diamond surface of FTIR-ATR by forceps, to investigate the polymer characters (Li et al., 2015). Furthermore, some MPs were identified using micro-Fourier Transformed Infrared Spectroscopy (FT/IR-6600 FT-IR made in Japan). The spectral resolution was in the range of 497.544 – 4003.5 cm^{-1} for the samples (*Fig. 6*).

Statistical analyses

In this study, microplastic particles refer to visually identified fibres, and there were few particles as plastic types (Baechler et al., 2020). Data collected on background

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characteristics of the sampled clams and microplastic particles were analysed in the Statistical Packages for Social Science (SPSS) version 20.0 and Microsoft Excel (Jeil et al., 2020) for means ± standard deviations, minimum, maximum, and percentages. Furthermore, to identify the differences in clam samples, types of variance (ANOVA) and post-hoc Tukey tests were analyzed (Hue et al., 2018a,b; Baechler et al., 2020). The models of linear regression were applied for observing the relationships between biological parameters (shell length, body wet weight) and MP load (per whole individual and g^{-1} tissue); the number of MPs per sample; g^{-1} tissue (wet weight; whole organisms) (Baechler et al., 2020). The significance was calculated for P ($P<0.05$). The statistical analyses were performed with the SPSS Statistic (Dong et al., 2018).

Figure 6. *FTIR spectrometer with ATR probe*

Results and discussion

Basic information on the sample clams

General sample clams

There are different sizes of clams in the study which are given as the mean ± SD such as clam_total_weight (17.12±6.23 g), clam_soft_tissues (4.13±2.45 g). Separately, the clam samples (10 clams altogether), four-locations and two year of age were illustrated in *Table 1*. The total weight of the soft tissues of clams in Quang Ninh (QN)-2-year was the highest, and Nam Dinh (ND)-1-year was the lowest, compared to others. The number of MPs per (g) was correlated with the clam's mean weight per (g) and clam's mean tissue weight per (g) on Spearman rank order correlations (rs) of 0.37 ($p < 0.05$), 0.36 ($p < 0.05$), respectively. Furthermore, the number of MPs was strongly correlated with MPs/clam's mean weight per (g) and MPs/clam's mean tissue weight per (g) 0.91 ($p < 0.01$), 0.71 ($p < 0.01$), respectively. The allometric relationship was disclosed between the clam's size and the MPs related to the considered clams as Saavedra et al. (2004) represented. Similarly, there was found to be a relationship between the rate of MPs/clam's size and the number of MPs as well (Narmatha Sathish et al., 2020).

Microplastic profusion in clam

The dispersion of MPs among the different ages of clams in the study is represented in *Table 2*. The highest variety of MPs was found in the clams of QN-2-year and the lowest abundance was found in ND-1-year. The number of clam bodies and MPs, varies from 0.25 ± 0.16 to 0.67 ± 2.98 items/ individual, from 0.11 ± 0.07 to 0.92 ± 0.59 items/g and from 0.02 ± 0.12 to 0.28 ± 0.13 items/soft tissues. However, Su et al. (2018) represented MPs extending from 0.4 to 5.0 items/individual (or 0.3 to 4.9 items/g) in

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Asian freshwater clams. In Quang Ninh, the young-sized clams contained a higher concentration of MPs (0.28 ± 0.13 items/soft tissues) than the older ones (0.25 ± 0.16 items/soft tissues). This was in contrast with Nam Dinh, where the older ones had a higher concentration of MPs. The level of microplastics was around one order of magnitude higher than those represented in mussels and oysters (Mathalon and Hill, 2014; Li et al., 2015). Furthermore, this might be growth of infiltration rate with the decline in the sampling size (Winter, 1974), which would increase in MPs in small-sized clams and decline in large-sized clams (Narmatha Sathish et al., 2020).

Table 1. *Mean values and range of measurements of sampled clams*

Location-age	Total mean clam weight (g)	Weight of soft tissues (g)
Quang Ninh-1-year	176.11±17.72	49.67±4.82
Quang Ninh-2-year	246.44±24.32	77.67±5.85
Nam Dinh-1-year	121.64±14.99	20.45±2.02
Nam Dinh-2-year	169.20±13.82	28.50±1.90

Table 2. *Mean values and range of microplastic and clam tissues*

Location-age	Number of miscroplatics *(Individual)*	Microplastic density/clam *(Individual)*	Microplastic /total weight *(g)*	Microplastic /tissues weight *(g)*
Quang Ninh-1-year	4.56±2.92	0.33±0.5	0.88±0.43	0.28±0.13
Quang Ninh-2-year	6.67±2.96	0.67±2.98	0.92±0.59	0.25±0.16
Nam Dinh-1-year	2.45±1.44	0.25±0.16	0.11±0.07	0.02±0.12
Nam Dinh-2-year	5.40±4.09	0.54±0.40	0.21±0.13	0.04±0.02

Futhermore, MPs/tissues were directly proportional to MPs/distance. Interestingly, younger clams (small-size) had an outstandingly greater concentration of MPs than the older ones (over medium-size). The highest rate of MPs/tissues and MPs/distance in 1-year-clams is 0.09 (ND) and 0.05 (QN) respectively (*Fig. 7*). However, according to research in India conducted by Narmatha Sathish et al. (2020) small-sized sample clams had higher concentrations of MPs. These fluctuations could be different according to the specific environments for the growing organisms (Cho et al., 2019).

Microplastics under digital microscope

Color of microplastics

The sample analysis showed MPs of various colors and sizes. Multiple colors of microplastics were recorded in clams, such as clear white, opalescent, blue, red, and black *(Fig. 8)*. The proportion of black colors was 55%, significantly larger than other colors. However, this finding was different from that of Lee et al. (2013), who reported that the transpearent MPs were the most commonly found category among all sample clams in China. The rate of clear-white, opalesent, and blue colors was around a mere 1%.

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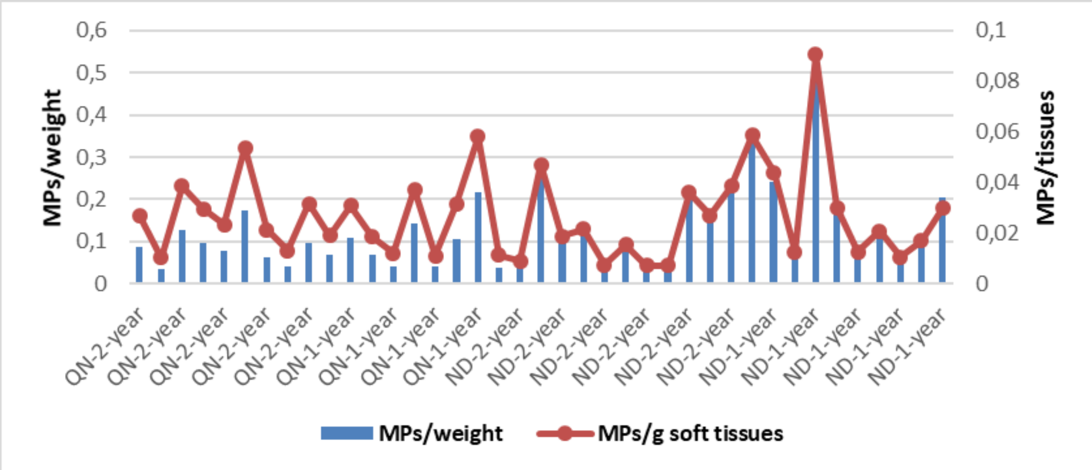

Figure 7. *The rate of MPs in sampling clam*

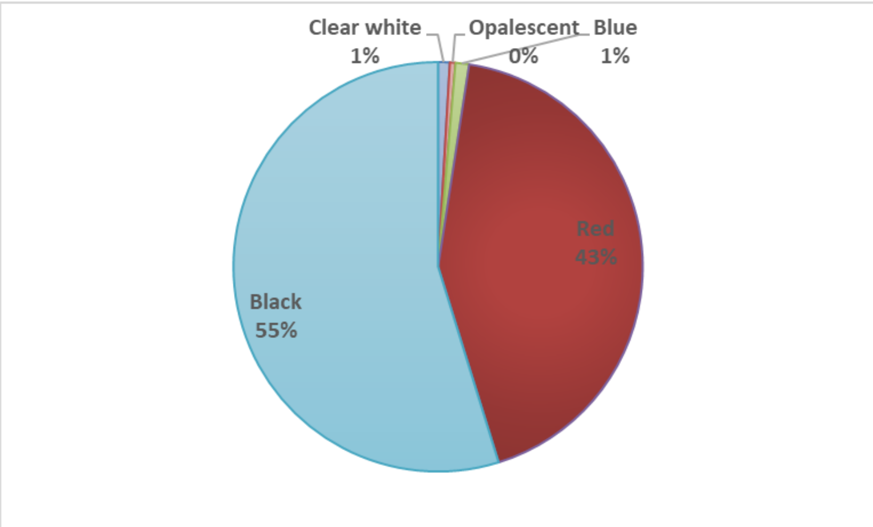

Figure 8. *Percentage of MPs colors from clams*

Shape of microplastics

Following clam shapes, there were three MP types such as fibers, fragments, and pellets. Fibers (68%) are the most common type of MPs in clams as reported by Narmatha Sathish et al. (2020) on the Tuticorin coast of the Gulf of Mannar (GoM), India (*Fig. 9*). Pellets were common in the sample clam but were not identified at all sampling locations. These research results were also supported by Li et al. (2015) when they reported the various MP types, including rich fibers in China. Davidson and Dudas (2016) also revealed that most commonly, fibers were observed in Manila clams. Additionally, De Witte et al. (2014) considered that fibers needed a longer time than other MPs to be eliminated. Further, Renzi et al. (2018) noted that the fibers found under gills and in the hepatopancreas could not be easily withdrawn.

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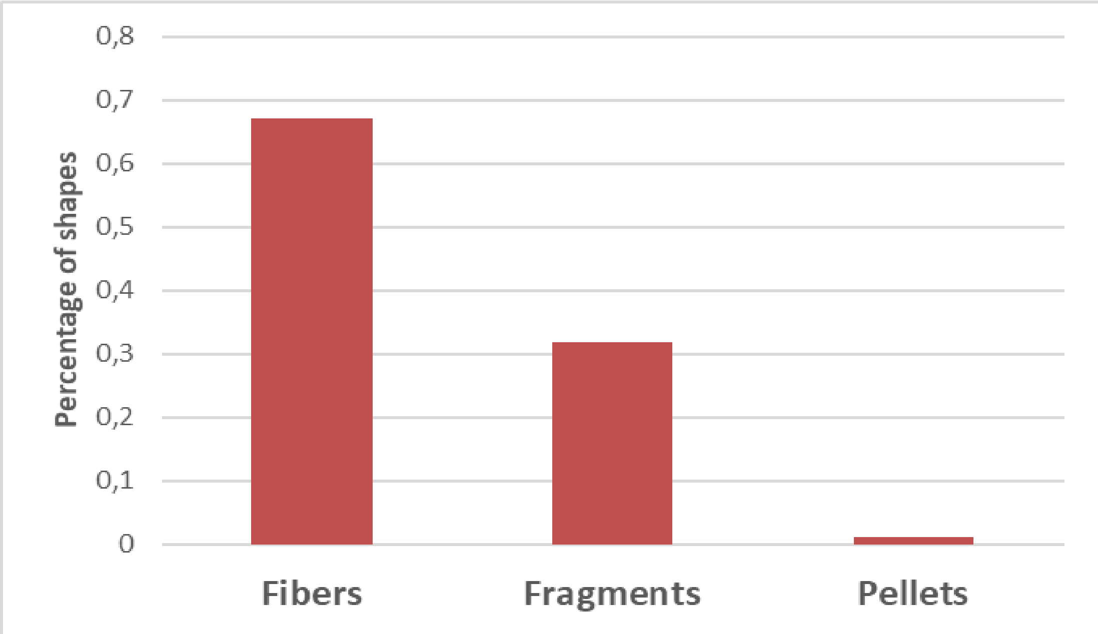

Figure 9. *The main abundance of microplastics shapes*

Size of microplastics

Interestingly, MPs were observed under Microscope (*Fig. 10*). The size of fibers (4.70±4.32 mm) and fragments (4.00±3.22 mm) were largest in ND-2-years and QN-2-years respectively, while both MP sizes were lowest in ND-1-year (*Table 3*). It is clear that the size of MPs in longer clams is greater than MP size in small ones which is similar to the findings of Narmatha Sathish et al. (2020), who considered that the direct proportionality of MP size to the size of clam body was significant. However, the overall size of MPs was different in QN-1-year (2.04±2.51mm), which was the biggest among others.

Table 3. *Mean values and range of microplastic sizes in different clams*

Location-clam-age	Microplastic size (mm)	Fibers (mm)		Fragments (mm)
Quang Ninh-1-year	2.04±2.51	2.56±2.24		1.89±1.05
Quang Ninh-2-year	1.50±1.80	3.44±2.24		4.00±3.22
Nam Dinh-1-year	1.49±0.78	2.10±1.29		1.00±0.00
Nam Dinh-2-year	1.61±1.05	4.70±4.32		1.17±0.41

Fig. 12 illustrates the spectrum of some resins found in clams at the two study sites, including Nylons in clams in Quang Ninh and unidentified resins, which are very diverse.

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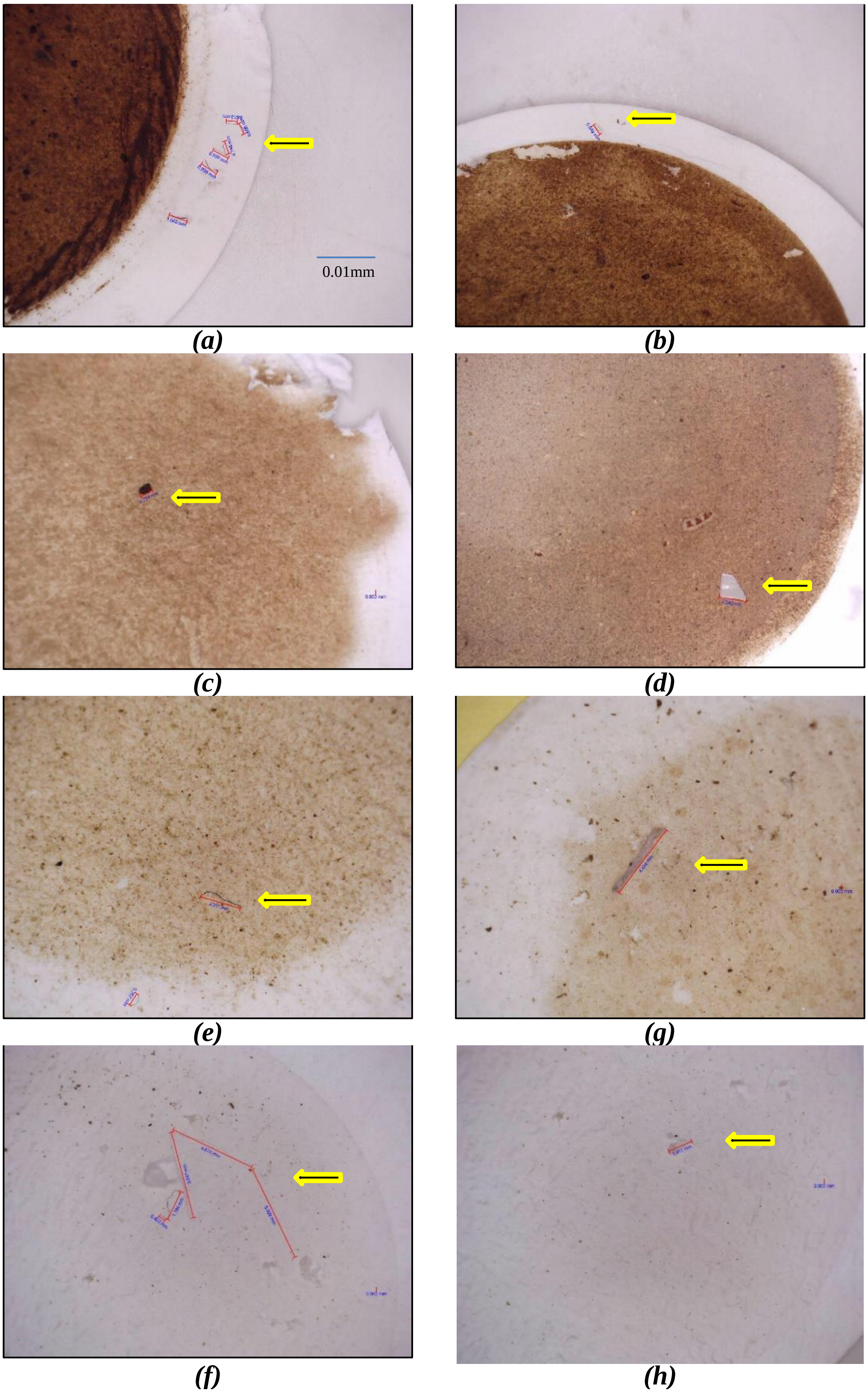

***Figure 10.** Photographs of different types of microplastics in clams from the case study. The photographs were taken directly on the filter paper of Quang Ninh (2-year (a, b), 1-year (c,d)); Nam Dinh (2-year(e,g), 1-year (f,h)) samples; Black yarn beads (a,c,e,f); Opaque white fragments (b,d,g,h); Fibers (a,b,e,g,f) Fragments (d,h), Pellets (c)*

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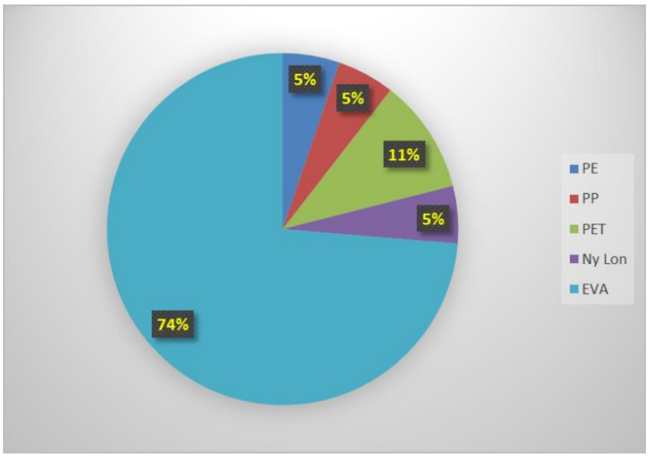

Figure 11. *The distribution of polymer composition of MPs in clam samples*

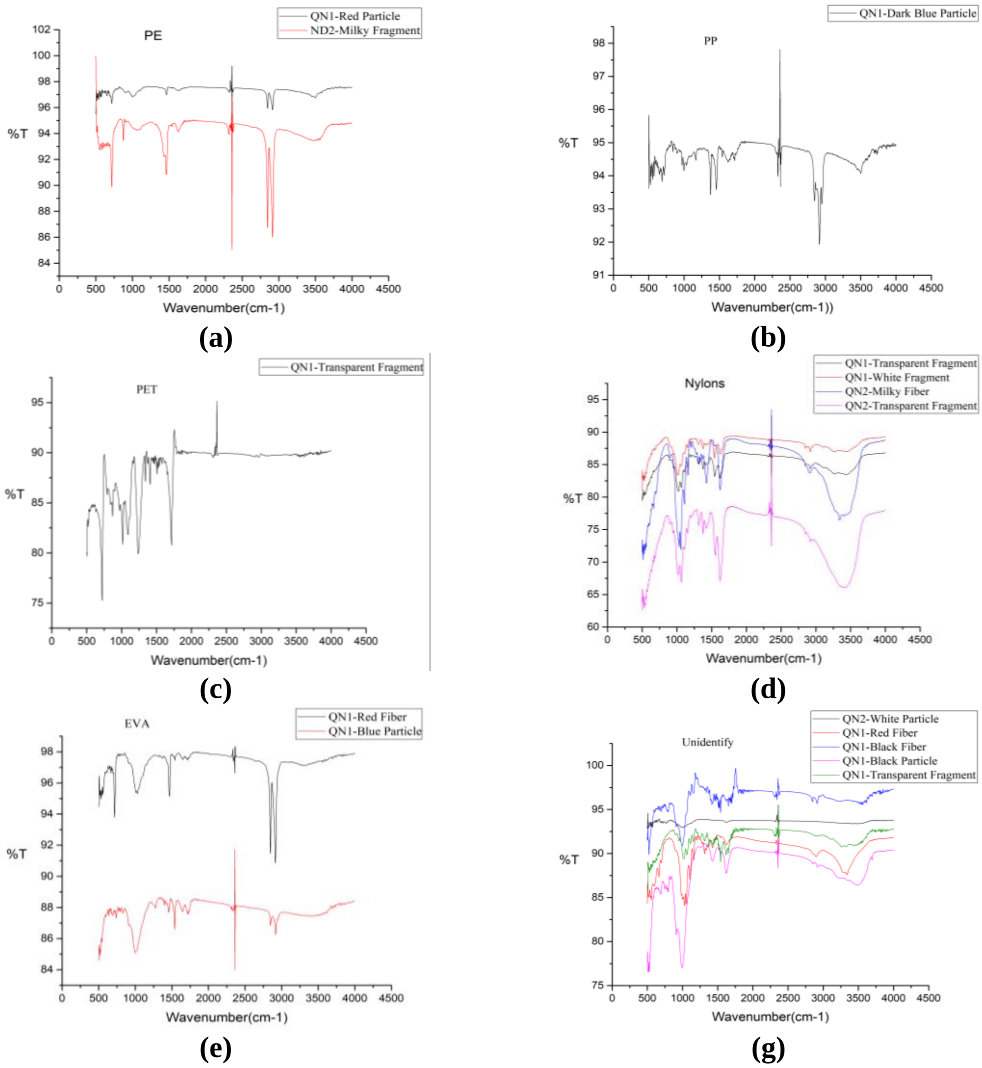

Figure 12. *Identification of different types of microplastic with micro-Fourier Transformed Infrared Spectroscopy (μ-FT-IR). The performed transmittance; a (PE: Polyetylen); b (PP: Polypropylene); c (PET: Polyethylene Terephthalate); d (Nylon); e (EVA: Ethylene vinyl acetate copolymer); g (identification)*

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Conclusion

This study used stereo microscopy and FTIR-ATR to explore the levels of contamination and characteristics of MPs in clam species (*Meretrix lyrata and Tapes dorsatus*) in two locations of common farming aquaculture in north Vietnam. The highest number of microplastics was found in the oldest clams. Similarly, the fiber types are the most common in the microplastic samples, thus fiber MPs should be considered in future environmental management. This research emphasized that the number of MPs is correlated with the clam's weight and soft tissues. Also, the clam is still a traditional food bivalve in humans and it will raise the risk of MP contamination through daily consumption (Narmatha Sathish et al., 2020). Further research investigating the impact of eating clams on human MP ingestion and its potential transfer through food consumption is needed. Furthermore, in this case, the question of correlation between microplastic pollution in clams and their living environment (such as waters, sediment, etc) should be considered, so environmental protection can be supported.

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Declaration of interest. The authors declare that there is no conflict of interests in this paper.

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