Effect of microplastic polystyrene on shrimps (*Neocaridina sp.*)

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1 Introduction

Many of products used every day are crafted from plastic polymers. Lot of them are collected and carried to junkyard but many leaks through rivers to oceans or end up in forests, meadows, fields, ... Complete decomposition of plastic material is very slow and take many years. During years of their existence small fragments are chipped away. These pieces are so small that organisms are not able to see them. Microplastic fragments than end up on fish gills and in other internal organs. Usually microplastic material is not dangerous for body of organisms. The problem is their ability to attract toxic compounds on their surface. These compounds can be released in different organs with fatal consequences. There are not many studies which describe behaviour of organisms exposed to contaminated environment with microplastic materials. In our study we were focused on microplastic fibres of polystyrene and its effect on shrimp behaviour: their average speed, distance moved and acceleration.

2 Microplastics

Microplastics are defined as small pieces of plastic. Fragments are less than 5 millimetres long and end up in environment as consequence of plastic pollution. Plastic can be lighter (polypropylene) or denser (acrylic) than seawater. Lighter plastics are floating on water level and often are accumulated on shorelines and water surface. Over time plastic objects are attracted by organisms and many particles adhere to their surface thus they can sink. Some studies shown that some items made from plastic sink to deep sea. Another study confirmed presence of microplastic pieces at low densities in deep sea. Microplastics are created from many products as cosmetics, plastic bottles and bags. During washing in washing machines, microplastics are created from fragments which are torn away from clothes made from nylon, spandex and other usually used plastic materials. Microplastics consist of hydrogen and carbon atoms bound together in polymer chains. Many other chemicals are present as well, as tetrabromobisphenol, polybrominated diphenyl ethers, phthalates and different chemical additives used in industry. We can divide microplastics to two groups: primary and secondary microplastics. Primary microplastics can be found in personal care products, plastic pellets (used in industrial manufacturing) and plastic fibres (used in synthetic textiles) in form of microbeads. Microbeads enter environment directly through product use, spills during transportation or manufacturing, during washing. Secondary microplastic are created from breakdown of larger plastics. Exposure to unfit weather as ultraviolet radiation, waves in seaside locations, strong wind and many other factors help with plastic breakdown. Microplastic materials are not biodegradable so they persist in environment and accumulate in water, soil but also in bodies of organisms. In early 21st century, in oceans were estimated 4 to 14 million tons of plastics. Microplastics were also detected in air, in dust and airborne fibrous particles. Small plastic fragments can enter body via inhalation, however effect on health is not unknown. In water ecosystems (marine and freshwater), microplastics had been found in more than 100 aquatics species. Plastics fragments have been found also in digestive tracts and tissues of many invertebrate sea animals. Birds and fish ingest light microplastics floating on water surface by mistake. Ingestion can cause many problems as less food consumption, neural and reproductive disorder. Microplastics were surprisingly found in human stool of every individual tested in pilot study.

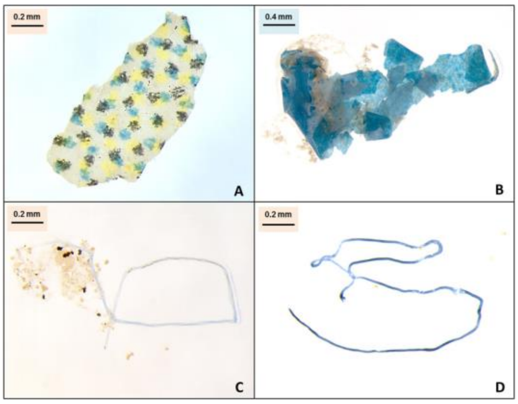

Figure 1 Examples of microplastics fragments (A), films (B) and fibers (C,D)

Typical hotspot for microplastic accumulation is gyres. Maximal concentration measured was 32,76 particles/m^3 and mass 250 mg/m^3 in North Pacific Subtropical Gyre. Great hotspots for microplastics are industrial coastal areas. In Swedish harbour area, concentrations of 100 000 particles/m^3 in seawater have been detected. Microplastics have been found on eighteen shores across whole world with tendency toward fibrous shape. Maximum concentration found in shore was 124 fibres/L.

The entanglement and ingestion of large plastic items can be seen around the world in vertebrates. More than 200 marine species are impacted by plastic ingestion. In 2012 it has been revealed that 80% incidents between organisms and marine debris was associated with macroplastics and 11% with microplastics.

Microplastics can be ingested by low trophic suspension, filter and deposit feeders, detritivores and planktivores. Accumulation in organisms may cause internal abrasions and blockages. Toxicity rise from leaching contaminants as plastic additives and monomers, which can cause carcinogenesis or endocrine disruption. On surface, hydrophobic persistent organic pollutants (with great affinity to hydrophobic surface of plastic) may be concentrated. Microplastics have large surface, thus they are easily contaminated (up to six orders of magnitude greater than seawater). Main factors affecting the bioavailability of microplastics are size, density, abundance and color.

The buoyancy is influenced by biofouling. Scientists observed that polyethylene bags developed biofilm within one week, which increased throughout three weeks. After only three weeks bags started to sink below sea surface. Fouled microplastics may sink, for example high-density plastic PVP (specific gravity 1,38). Created fragments may be found in different depths and available to benthic suspension and deposit feeders, detritivores and other organisms, eventually reaching the benthos as they sink.

Colored microplastic may resemble color of prey, so they can be ingested by mistake by predator. Some commercially important fish and their larvae are eye-dependent predators eating zooplankton and may feed on white, tan or yellow microplastics. Studies in New England support this statement. In Niantic Bay area, fish ingested only opaque, white polystyrene spherules.

Studies shown that perfluorooctanesulfonate is adsorbed on PE and PS under high salt concentration. perfluorooctanesulfonate and perfluorooctanesulfonamide sorption on PVC and PE were stronger that that of PS. This statement indicates that PE and PVC accumulate more perfluorochemicals than that with PS.

Fluoranthene is heavily adsorbed on micro-PS surface. One study tested possibility of enhancement by plastic and micro-algae. Results shows that there was no effect on fluoranthene bioaccumulation. Despite this fact micro-PS may modify bioavailability and fluoranthene kinetics in mussel tissues. Micro-PS triggered substantial modulation of cellular oxidative balance, an increase in histopathological damages, percentage of dead hemocytes and lysozyme mRNA levels, which suggested an impairment of the bivalve metabolism upon micro-PS exposure.

Effect of polystyrene have been tested on Pacific oyster. Micro-PS with diameter ranged from 2 to 6 μm witch concentration 0,023 mg/L was used. Oysters were exposed to Micro-PS for two months during reproductive cycle. Results shown that oysters consumed more 6 μm micro-PS particles than 2 μm particles. Microalgae consumption and absorption efficiency were higher for exposed oysters. Scientists suggest compensatory and physical effects on both digestive parameters. After two months from exposure, oysters had significant decreases in sperm velocity (-23%), diameter (-5%) and oocyte number (-38%). The D-larval yield and larval development of offspring derived from exposed parents decreased by 41% and 18%, compared with control offspring. Exposed oysters also shown signs of endocrine disruption, however no endocrine disruptors were found.

Different microplastics polystyrene diameters ranged from 50 nm to 10 μm was tested for phenanthrene bioaccumulation in *Daphnia magna*. Particle with diameters 50 nm were toxic and damaged physically *D. magna*. Combination of 50 nm particles and phenanthrene showed higher toxicity than other tested diameters. During fourteen-day incubation time, presence of microplastic particles enhanced bioaccumulation of phenanthrene-derived residues in *D. magna* bodies. Microplastics also inhibited dissipation and transformation of phenanthrene in medium. Particles with 10 μm diameter did not show significant effect on bioaccumulation, transformation and dissipation of phenanthrene.

Figure 2 Fluorescent microplastics found in D. magna

Commercially important crustacean, *Crangon crangon*, have been exposed to microplastics. Used microplastic fibers ranged from 200 μm to 1000 μm. Presence of microplastics have been confirmed in 63% of the assessed shrimp with average value of 0,68±0,55 microplastics/g w. w. (1,23±0,99 microplastics/shrimp) was obtained for shrimp in the sampled area. Uptake of microplastics were higher during October during March, which confirms that temperature is one of the key factors in microplastics uptake. Results also suggests that microplastics with diameter more than 20 μm are not able to translocate into the tissues.

Eleven different microplastic particles with different shapes (fibers, spheres, fragments) were tested on grass shrimp *Palaemonetes pugio*. Microplastics were made from polyethylene, polypropylene and polystyrene. Shrimps were exposed to particles with diameters ranged from 30 to 180 μm with concentration 50 000 particles/L for three hours. Spheres and fragments under 50 μm were not acutely toxic. Mortality was higher in experiments with fibers with 93 μm diameter. Results confirmed that shape of microplastic has a key role in number of particles ingested by a shrimp. The residence time of particles in the gut ranged from 27-75 hours. Within the gills, the residence time ranged from 27-45 hours. Mortality ranged from 0% to 55%.

One study picked up ecologically and economically key species in Mediterranean Sea, *Aristeus antennatus*. Scientist confirmed occurrence of microplastics in 39,2 % of the tested individuals. In arears closer to Barcelona, 100% of tested shrimps were contaminated with microplastics. Microplastics were found in shrimps living in depth ranging from 630 to 1870 m. Study revealed that shrimp affected by microplastics had a higher presence of endobenthic prey. It is interesting that microplastics had no negative effect on biological condition of shrimps. Microplastics had form of fibers or tangled fibers in balls. Three different polymers were found: polyethylene terephthalate (57,1%), nylon/polyamide (28,6%) and rayon/viscose (14,3%).

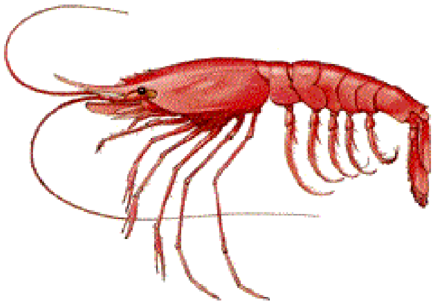

Figure 3 Aristeus antennatus

Scientist tested effect of 40 nm anionic carboxylated (PS-COOH) and 50 nm cationic amino (PS-NH_2) polystyrene nanoparticles on larvae of *Artemia franciscana*. No signs of mortality were confirmed but several sub-lethal effects were evident. Concentrations of PS-COOH ranging from 5 to 100 µg/ml resulted massively sequestered inside the gut lumen of larvae, probably limiting food intake. Some were lately excreted but not a full release was observed. PS-NH_2 with concentration from 5 to 100 µg/ml also accumulated in larvae. Plastics were adsorbed at the surface of sensorial antennules and appendages limiting larvae mobility. Shrimps exposed to PS-NH_2 undergo multiple molting events during 48 hours of exposure.

Microplastics can reach the bottom of the sea and oceans, affecting the life of local organisms. Samples have been taken from multiple locations and depths. In Southern Ocean (depth 2749 m) have been found microplastic fragment 118 µm long and 60 µm, wide. In Nile Deep Sea Fan (depth 1176 m) have been found microplastic 75 µm long and 53 µm wide. Lastly in Porcupine Abyssal Plain in depth 4842 meters have been found plastic particles of different shapes and sizes. First was 161 µm long and 137 µm wide, second 83 µm long and 44 µm wide and third 125 µm long and 76 µm wide. In the deep sea microplastics can reach an average abundance of 0,5 microplastic per 25 cm^2 in the top centimeter of sediment. The highest concentration was observed in sediment from Porcupine Abyssal Plain (1 particle per 25 cm^2).

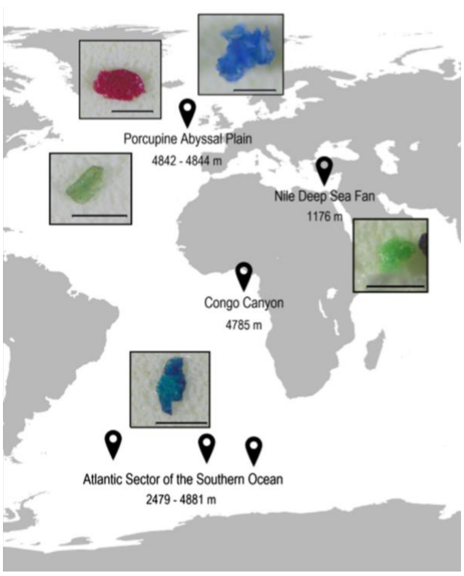

Figure 4 Microplastics found in sediments across the world

Surprisingly many microplastics have been found in Belgian coastal waters. Four different types of particles were detected: fibers (59%), granules (25%), spherules (12%) and plastic films (4%). The fibers have been identified as polypropylene, nylon and polyvinyl alcohol. Plastic films were made from nylon and granular pieces from polypropylene, polyethylene and polystyrene. In the harbors were found 390 particles per kilogram of dry sediment, which is 15-50 times higher than reported maximum concentrations of other, similar study areas.

Significant concentrations of microplastics have been found in sediment of St. Lawrence river. Samples were retrieved from 10 freshwater sites along a 320 km section from lake St. Francis to Quebec City. Found microplastics had form of polyethylene microbeads with diameter ranging from 0,40 to 2,16 mm. The highest site density was $1{,}4 \times 10^5$ microbeads/m^2 (10^3 microbeads/L). Similar concentrations can be found in most contaminated marine sediments. Median and mean densities across sites were 52 microbeads/m^2 and 13 832 (±13 677) microbeads/m^2. Diameter of microbeads was smaller at sites receiving municipal/industrial effluent (0,70±0,01 mm) than at non-effluent sites (0,98±0,01 mm).

3 Experiments and results

Experiments were held under laboratory temperature and pressure. 126 hundred shrimps (*Neocaridina* spp.) have been split equally to nine tanks (5 liters each). We added microplastic polystyrene (diameter <0,5 mm) of concentration 40 mg/L into six tanks. Every third day, 2/3 of water has been changed. Movement activity of shrimps (3 from each tank) and distance were measured 7th and 14th day of contamination. For recording we used GoPro Hero 7, recording time has been set to five minutes. Data has been evaluated by program made by Loligo Systems, which uses RGB spectrum for movement scanning.

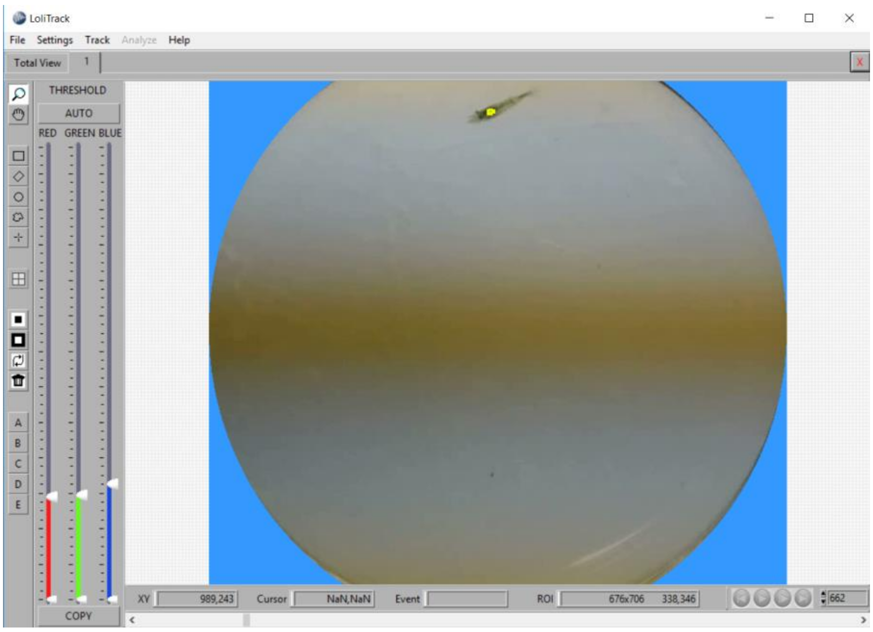

Figure 5 Measuring shrimp movement with Loligo Systems

After seven days from contamination we observed, that exposed shrimps were slower, they were less active and moved less distance than control units. However, after fourteen days from contamination exposed shrimps were faster, more active and moved more distance than control units. We also compared exposed and control shrimps between themselves after 7th and after 14th day from exposition. After 14 days control units were much slower, less active and moved less distance then shrimps measured 7th day. Surprisingly exposed shrimps after 14 days were faster, more active and moved more distance than shrimps exposed to microplastics for 7 days. Detailed results and percentual comparison can be seen in table 1 and 2.

Table 1 Comparison of selected parameters between control and exposed units

Parameter	Control/Exposed units (7 days)	Control/Exposed units (14 days)
Velocity	-1,5 %	+64,4%
Acceleration	-17,4%	+97,8%
Active time	88,8%/88,0%	83,2%/88,1%
Inactive time	11,2%/12,0%	16,8%/11,9%
Distance moved	-2,8%	+74,0%

Table 2 Comparison of selected parameters in 7th and 14th day

Parameter	Control units (7/14 days)	Exposed units (7/14 days)
Velocity	-44,8%	+15,3%
Acceleration	-74,4%	+33,1%
Active time	88,8%/83,2%	88,0%/88,1%
Inactive time	11,2%/16,8%	12,0%/11,9%
Distance moved	-53,8%	+16,3%

4 Conclusion

Nowadays, there are lot of studies about microplastics contamination in environment. Some scientists examine sediments, water or air pollution; some are interested in microplastics in food chain or effects on respiratory and digestive organs. We were concerned about microplastics effect on movement and activity of shrimps. At first exposed shrimps were slower and less active than controlled units, but after 14 days from exposition exposed shrimps were faster and more active than control units. After 14 days, control units were much slower and moved less distance than control units during 7th day. This phenomenon may be caused by stress, adapting to new environment and overeating. Exposed shrimps were faster, slightly more active and moved more distance after 14 days from exposition than shrimps exposed to microplastics for 7 days. This may be caused by adsorbing microplastics particles on limbs or body and contamination by microplastics may put shrimps under stress. Contamination of respiratory and digestive organs by micro-PS may irritate shrimps to move faster with higher acceleration. Shrimps could mistakenly eat accumulated micro-PS in excrements, which may lead to satiety feeling. Mortality was not observed in tanks with control and exposed units, confirming fact that selected micro-PS concentration (40 mg/L) is not acutely toxic for shrimps of *Neocaridina* species. Further study is needed to examine microplastics effect on shrimp respiratory and digestive organs.

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