**Bailey Levesque**
bailey.levesque@nrc-cnrc.gc.ca
Halifax, Nova Scotia, Canada

NRC·CNRC

Zebrafish Toxicology, Neurobiology, and Genomics, Aquatic Crop Resource and Development

# Bioaccumulation of various nanoplastic particles in larval zebrafish (*Danio rerio)*

Bailey Levesque[1], Lee Ellis[1], and Anja Vogt[2]
[1] National Research Council of Canada, Halifax, NS
[2] National Research Council of Canada, Charlottetown, PEI

## Abstract

Plastic particles have been found present in almost all aquatic environments worldwide yet the specific threats they pose to both marine and freshwater biota remains unclear. Research on the toxicity of these particles has increased in recent years and has revealed that at environmentally relevant concentrations nanoplastic particles are taken up by aquatic organisms which can result in various toxic effects. Here, the impact of exposure to various sized nanoplastics was assessed using standard high throughput Zebrafish larval exposure paradigms. Based on the fish embryo toxicity (FET) model and general and behavioural toxicity assay (GBT), larvae were exposed from 6-120 hours post fertilization (hpf) and 72-120 hpf respectively. The models were used to assess acute toxicity along with the bioaccumulation and excretion of 40-60 nm and 100 nm polystyrene nanoplastic particles. The analysis of particles via fluorescent microscopy confirmed their uptake and accumulation within the larval zebrafish as well as their subsequent excretion. This testing provides insights into the ability of the larvae to uptake nanoplastics which will allow a more in-depth analysis of their potential toxic effects on zebrafish larvae and other aquatic biota.

## Methods

Zebrafish were exposed to Fluorescently labelled (Nile Red) nanoplastic particles with diameters of 40-60 nm and 100 nm. For the fish embryo toxicity assay (FET), larvae were exposed to nanoplastic particles from 6 to 120 hpf. Larvae were scored for toxic phenotypes at 72, 96, and 120 hpf. For the general and behavioural toxicity assay (GBT), larvae were exposed from 72-120 hpf and scored for toxic phenotypes at 96 and 120 hpf. Behaviour was analyzed at 120 hpf for the GBT assay. Fluorescent microscopy was conducted for the FET assay at 72, 96, and 120 hpf.

## Results

There were no visible toxicological phenotypes at any exposure concentrations for larvae exposed to either 40-60 nm or 100 nm particles. Behaviourally, larvae exposed to 40-60 nm particles at concentrations of 0.2, 0.1, 0.07, and 0.05 µg/ mL travelled significantly more distance in the first 30 minutes of light for the GBT assay compared to the controls. During the first light to dark transition only the larvae exposed to the 40-60 nm particles at 0.2 µg/mL showed a significant difference from the control. There were no significant changes in behaviour observed for the 100 nm exposed larvae. Fluorescent images of larvae at 72, 96, and 120 hpf confirmed uptake of 40- 60 nm particles and subsequent excretion at all exposure concentrations. Uptake and excretion were not confirmed for the 100 nm polystyrene particles.

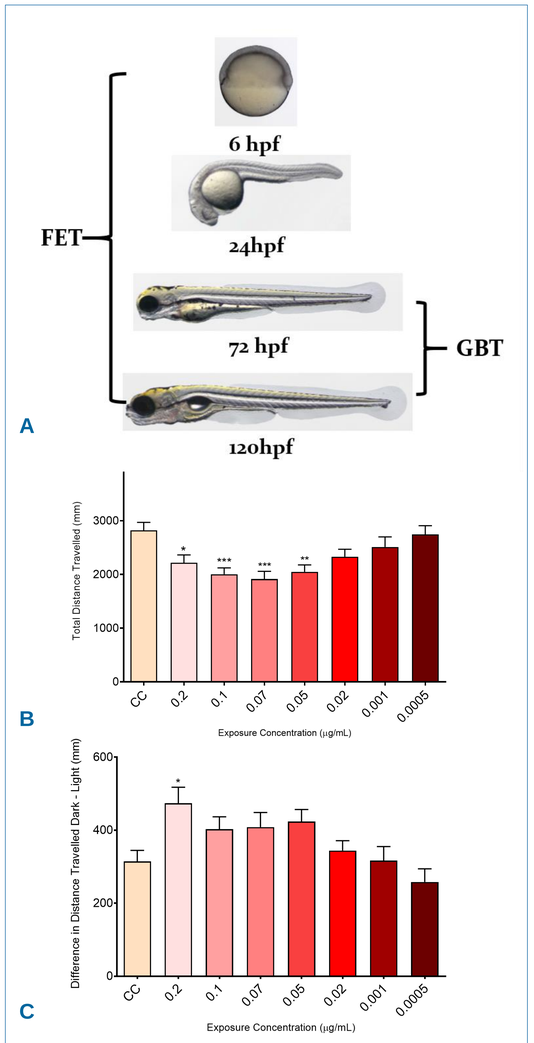


**Figure 1.** Visual representation of the FET and GBT assays (A). Larval activity response for 30 minutes in a lit environment (B) and a 5 minute dark- light transition period (C) following exposure to polystyrene 40- 60 nm nanoparticles from 72-120 hours post fertilization (hpf). Error bars represent the standard error of the mean. Asterisks (*) indicate a statistically significant difference in activity compared to the controls using a one way ANOVA with a Sidak multiple comparisons test.

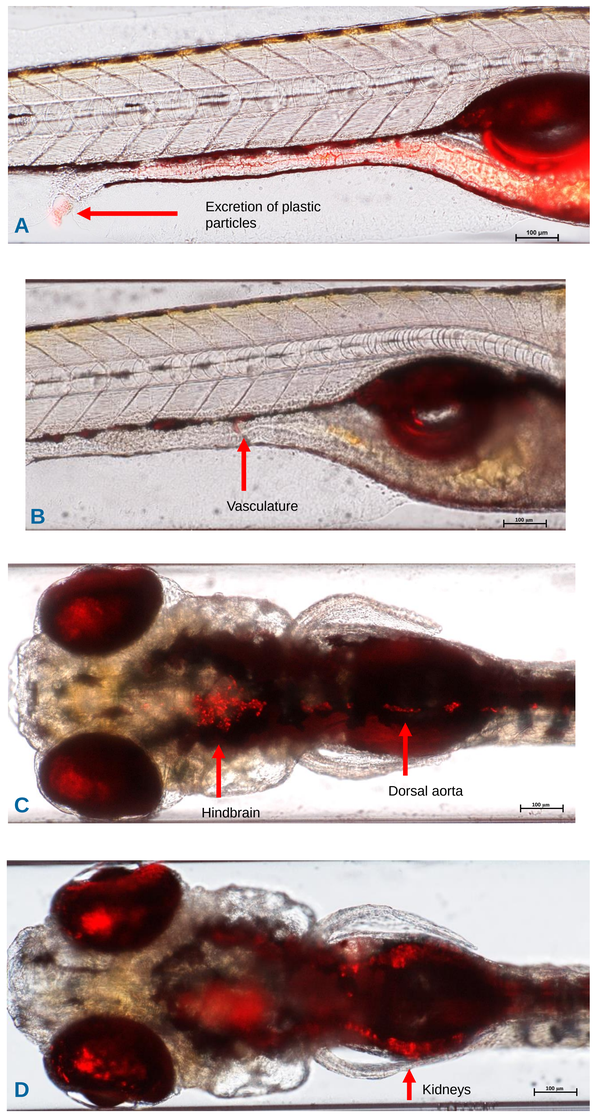


**Figure 2.** Zebrafish larvae imaged at 120 hpf following a FET exposure of 40- 60 nm polystyrene particles at concentrations of 0.04 µg/mL (A), 0.02 µg/mL (B), 0.0005 µg/mL (C), and 0.01 µg/mL (D). Arrows indicating area of accumulation are shown.

## Discussion

Previous studies of similar sized nanoparticles did not observe toxicological phenotypes at similar concentrations [5,8]. However, toxicological phenotypes were seen when exposure concentrations were increased to levels higher than those in this study [2,3,6] and to a level that is not interpreted as environmentally relevant [7]. Alterations to normal behaviour in larval zebrafish following plastic particle exposure has been previously documented. Contrary to our results, other studies found an overall decrease in movement during behaviour assays [3,8,9,10] when zebrafish were exposed to plastic particles. Differences in the results may linked to the size of larger particles and/ or higher exposure concentrations than those used in this study. This is supported by the differences found for the effects on behaviour between the 40- 60nm and the 100 nm exposed larvae, which may also be attributed to particle size differences. Increasing toxicity with decreasing plastic particle size has been previously reported [4, 7].

## Conclusion

Nanoplastic particles are taken up by zebrafish larvae as early as 72 hpf and are subsequently excreted. Behavioural assays show the presence of sub- phenotypic effects, including a general increase in baseline behaviour, at the highest nanoplastic concentration tested. Elucidating the effects of various plastic particles on fish is integral as their ubiquitous presence in the environment presents potential effects at the individual, population, and ecosystem level.

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