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1 Detection, biophysical effects and toxicity of polystyrene nanoparticles to the
2 cnidarian *Hydra attenuata*

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9 Abstract

10 The occurrence of nanoplastic particles (NPs) in the environment has raised concerns about the
11 ecotoxicological risk to aquatic ecosystems. The purpose of this study was to examine the
12 bioavailability and toxicity of 50 and 100 nm transparent polystyrene NPs to the cnidarian *Hydra*
13 *attenuata*. The hydra were exposed to increasing concentrations of 50 and 100 nm NPs (1.25, 2.5,
14 5, 10, 20, 40 and 80 mg/L) for 96 h at 20°C followed by a 24h-depuration step. Hydra were
15 analysed for morphological changes, bioaccumulation of NPs using a novel assay for polystyrene
16 NPs, oxidative stress (lipid peroxidation), polar lipids, lipid-like liquid crystals (LCs) and viscosity
17 changes in the post-mitochondrial fraction. The results revealed that the organisms accumulated
18 detectable amounts of NP in a concentration dependent manner for both the 50 and 100 nm NP
19 that persisted after 24 h in clean media. Changes in morphology were observed with a 50% effect
20 concentration of 3.6 and 18 mg/L for the 50 and 100 nm diameter NPs respectively. However
21 based on the particle density, the 100 nm proved to be 1.7 times more toxic than the 50 nm NPs.
22 Exposure to NPs led to decreased biomass, lipid peroxidation (LPO), increased polar lipids levels,
23 viscosity and formation of LCs at the intracellular level. In the more toxic NP (100 nm), NPs in
24 tissues were correlated with LCs, polar lipids and LPO levels. It appears that the formation of
25 organized LCs and polar lipids of NPs in cells was involved with NPs toxicity and could represent
26 a yet unidentified, detoxifying/bioactivation mechanism against colloidal plastics in cells. In
27 conclusion, NPs are bioavailable to hydra and lead to LPO and lipid mobilisation in hydra. The
28 capacity of increasing lipid mobilization and LCs could determine the size-dependence toxicity of
29 NPs.

30 Key words: nanoplastics, polystyrene, hydra, oxidative stress, neutral lipids.

31 Introduction

32 Plastic products are ubiquitous in the environment as they permeate many aspects of our daily
33 lives. They can be found in various products such as coatings, cosmetics, wiring, packaging, film
34 covers, bags and miscellaneous containers (Al-Salem et al., 2009). Although many plastics are
35 recycled, they are unfortunately still found in most water bodies including rivers and oceans

36 persisting for decades in the environment. Plastic particles could degrade in the environment from
37 abiotic (physical abrasion) and biotic factors leading to microplastics (operationally defined as
38 materials between 1 μm to 5 mm) and nanoplastics (NPs; 1 to 100 nm) (Lambert and Wagner,
39 2016). The long-term degradation of plastic materials will lead to staggering quantities of NPs in
40 the environment. Polystyrene-based plastics are one of the most abundant forms of plastics
41 representing circa 10% of total plastic production (Verschcoor et al., 2017) and can remain in the
42 environment for hundreds of years (Ho et al., 2018). Although microplastic materials can clog and
43 damage the digestive system and skin in birds and aquatic animals, the small size of NPs makes
44 them permeable to tissues and cells whereas they can accumulate and produce unknown effects at
45 the intracellular/macromolecular levels. Indeed, internalized NPs were shown to damage cells by
46 apoptosis, protein denaturation/fibrillation and the formation of organized lipophilic liquid crystals
47 (LCs) in cells (Colvin and Kulinowski, 2007; Wang et al., 2013; Gagné et al., 2019a). Although
48 several studies can be found on the effects of NPs in marine organisms, there are a limited number
49 of studies on the toxicity of NPs in freshwater invertebrates.

50 The freshwater hydranth *Hydra attenuata* is commonly found in freshwater ecosystems and is a
51 well-recognized test organisms in aquatic toxicity studies (Blaise and Kusui, 1997; Pascoe et al.,
52 2003; Quinn et al., 2012). The hydra is a simple organism composed of a tube-like structure, with
53 a foot attaching to a solid substrate and a head composed of 4-6 long tentacles for food capture.
54 The toxic intensity (reversible sub-lethal to irreversible lethal effects) can be conveniently
55 observed through a stereomicroscope by a series of morphological changes such as antennae
56 shortening, loss of antennae and compressed tube. More recently, the hydra was used to examine
57 the toxicity of both microplastics (Murphy and Quinn, 2018) and nanoplastics (Gagné et al.,
58 2019b). Hydra were able to ingest small microplastics fragments from face wash soaps that

59 compromised feeding activity and displayed sub-lethal stress by regression of antenna. These
60 studies revealed that microplastics could reduce feeding activity whereas NPs could significantly
61 induce reversible morphological changes and increase polar lipids levels in hydra. In the case of
62 polystyrene NPs, the styrene-based surface provides a hydrophobic environment (aryl benzene)
63 which could induce strong hydrophobic interactions given the high surface area of nanoparticles.
64 It is hypothesized that the introduction of the hydrophobic surface of NPs could elicit changes on
65 lipid mobilization and other macromolecules such as less polar proteins/peptides at the surface of
66 the NPs.

67 The purpose of this study was therefore to examine the bioavailability and toxicity of 50 and 100
68 nm polystyrene NPs in *Hydra attenuata*. The bioavailability of NPs was determined by a novel
69 fluorescent probe methodology. Toxicity effects were determined by both morphological changes
70 and sublethal biomarkers. The latter consisted in changes in lipid peroxidation, polar lipid levels,
71 microviscosity changes and the formation of anisotropic LCs. The mode of action based on
72 hydrophobic interactions of NPs was examined in hydra exposed to NPs.

73 74 **Materials and Methods**

75 76 ***Preparation and exposure of NPs to hydra***

77 Transparent, unlabeled polystyrene nanoplastics (NP) of 50 and 100 nm diameter were obtained
78 from Polyscience Inc (USA). The hydra were maintained in a media consisting of 1 mM CaCl₂
79 and 0.5 mM TES (N-tris [hydroxymethyl]methyl 1-2- aminoethanesulfonic acid), pH 7.0 at 20°C
80 as previously described (Blaise and Kusui, 1997) and were fed each day with preparations of live
81 *Artemia salina* brine shrimps. Exposure tests were conducted in 12-well microplates with three
82 animals placed in each well (n=9 hydra per treatment) containing 4 mL of media. The NP
83 suspension was prepared at 0.5 g/L in MilliQ water to prevent aggregation, with the final dilutions

84 prepared in hydra media. The size distribution of these NPs were examined in MilliQ water and in
85 the incubation media using dynamic light scatter instrument with an operating laser at 532 nm
86 (Mobius Instrument, Wyatt Technologies, Santa Barbara, CA, USA). Unexposed hydra
87 maintained in culture media only served as controls. The exposure concentrations used were 0,
88 1.25, 2.5, 5, 10, 20, 40 and 80 mg/L corresponding to 0.32, 0.64, 1.29, 2.57, 5.15, 1.03, 2.06×10^{12}
89 nanoparticles/L for the 50 nm NP and 0.04, 0.079, 0.159, 0.318, 0.636, 1.27, and 2.54×10^{11}
90 nanoparticles/L for 100 nm NP respectively. Hence, on a mass basis, 50 nm NP contains 8 times
91 more particles than the 100 nm NP. The selected concentration were chosen to attain a lethal
92 concentration which was in ppm range. These concentrations are not necessarily environmentally
93 realistic but could be attained in a spillage situation (e.g. sediments contaminated by miscellaneous
94 plastics compounds released by a nearby industry or source).

95 After the exposure period (96 h), changes in hydra morphology were determined under a 6 x
96 stereomicroscope based on the Wilby scale (Wilby, 1989). Normal control animals maintained a
97 normal size with long and slender tentacles; sub-lethal (reversible) effects consisting of hydra
98 showing clubbed or reduced tentacles length and lethal (irreversible) effects consisting in shrunken
99 hydra at the tulip or disintegrated stages. Representative examples for each morphological changes
100 are presented in Figure 1. The number of hydra with morphological changes was the variable used
101 to estimate the 50 % effects concentration (EC50) and the 50% lethal concentration (LC50). The
102 positive control consisted of ZnSO₄ (LC50 of 1 mg/L; 0.7-1.3 mg/L 95% confidence interval)
103 which was used for quality assurance control. A subset of hydra at the low exposure group and
104 controls (0, 1.25, 2.5 and 5 mg/L) were allowed to depurate in clean media for 24 h before freezing
105 to determine the elimination potential of the NPs and persistence of toxicity. At the end of the
106 morphological assessment, the hydra media was removed from the wells, the hydra washed in 1

107 mL of media, detached and suspended in 250 μ L homogenisation buffer consisting of 50 mM NaCl
108 containing 10 mM Hepes-NaOH, pH 7.4, 0.1 mM dithiothreitol and 0.1 μ g/mL apoprotinin
109 (protease inhibitor). Samples were stored at -85°C for NP uptake and biomarker analyses. The
110 hydra were homogenized using an hand-held pestle tissue grinder (Canlab, ON, Canada). The
111 homogenate was allowed to settle on ice for 30 min. Total protein contents in the homogenate were
112 determined using the Coomassie Brilliant Blue methodology in clear 96-well microplates
113 (Bradford, 1976). Serum bovine albumin was used for calibration.

114 *Detection of NPs in hydra*

115 Detection of polystyrene NPs was performed using the molecular rotor probe methodology
116 (Gagné, 2019). The probe consisted of a 10 mM stock solution of 9-(dicyanovinyl)-julolidine
117 (DCVJ) in ethanol, diluted to 10 μ M in MilliQ water on the day of analysis. For the assay, 20 μ L
118 of the homogenate was mixed with 180 μ L of 10 μ M of DCVJ probe in 96-well dark microplate.
119 Fluorescence was measured at 620 nm and 520 nm emission at 450 nm excitation for NP and
120 viscosity measurements respectively. Standard solutions of polystyrene NPs at 50 nm diameter
121 (0.05 and 0.1 μ g/mL) were prepared for instrument calibration where 1 RFU corresponds to 1
122 μ g/mL NP. The data was expressed a relative fluorescent units-RFU /mg proteins.

123 *Early biological effects of NPs*

124 The viscosity of the homogenate was determined by the DCVJ probe at the reported emission
125 wavelength (520 nm emission; 450 nm excitation) for viscosity assessment (Haidekker et al.,
126 2001). The viscosity of a media could be defined as the resistance of molecules to movement in
127 space at a given temperature. The DCVJ is a molecular rotor probe where energy is dispersed by
128 either by kinetic rotation or by fluorescence. As the viscosity increases, rotation is limited and

129 fluorescence increases to compensate this restriction. A volume of 20 μL of the homogenate was
130 mixed with 180 μL of DCVJ probe as described above. Standard solutions of glycerol were used
131 for calibration for instrument calibration where 1 RFU corresponds to 10 $\mu\text{g}/\text{mL}$ of glycerol. The
132 formation of dissipative structures such as liquid crystals (LCs) in the homogenate was determined
133 using fluorescence polarisation spectroscopy (Gagné et al., 2019a). The hydrophobic fluorescent
134 octadecyl ester (FOE) probe was used to detect the formation of LCs in hydra exposed to NPs. The
135 FOE probe was dissolved in dimethylsulfoxide at 1 mM concentration and kept at room
136 temperature in the dark. On the day of analysis, the FOE probe was diluted at 10 μM in 10 mM
137 KH_2PO_4 and HEPES-NaOH, pH 7.4, containing 140 mM NaCl. A 20 μL volume of the homogenate
138 was mixed with 200 μL of 10 μM FOE and fluorescence polarization was determined at 485 nm
139 excitation and 520 nm emission. Fluorescence polarisation was calculated by determining the ratio
140 between the perpendicular and parallel direction of the emission signal (perpendicular-parallel
141 fluorescence / perpendicular+parallel fluorescence) using dichroic mirrors (Synergy-4, Biotek
142 instrument, USA). Standard solutions of 50 nm NPs (0.05, 0.1 and 0.15 $\mu\text{g}/\text{mL}$) were prepared
143 since the polarization was shown to be concentration dependent in the presence of homogenate
144 extract (Gagné et al. 2019a). The fluorescence polarization data were imported to an Excel
145 Spreadsheet and analysed with SYSTAT software (version 13.2; USA). Fluorescence polarization
146 was measured in the same amount of homogenate proteins (10 $\mu\text{g}/\text{mL}$).

147 Lipid peroxidation (LPO) was determined in hydra homogenates using the thiobarbituric acid
148 method (Gagné, 2014) using a miniaturized adaptation for small volumes in 384-well microplates.
149 A volume of 10 μL of the homogenate was mixed with 20 μL of 10% trichloroacetic acid
150 containing 1mM FeSO_4 and 10 μL of 0.7% thiobarbituric acid in 384 wells dark microplates. The
151 mixture was placed at 75 $^\circ\text{C}$ for 10 min, cooled at room temperature before taking fluorescence

152 measurements. Fluorescence readings were taken at 540 nm excitation and 600 nm emission using
153 standard solutions of tetramethoxypropane (stabilized form of malonaldehyde) for calibration in
154 the blank media (homogenization buffer only). Results were expressed as μg thiobarbituric acid
155 reactants (TBARS)/mg total proteins in the homogenate.

156 The polar fraction of lipids in the homogenate was determined using the Nile red methodology
157 (Greenspan and Fowler, 1985). Nile red was prepared as 10 mM in methanol and diluted to 10 μM
158 in MilliQ water. A 20 μL volume of the homogenate was mixed with 180 μL of Nile red probe.
159 After 10 min, fluorescence was measured at 500 nm excitation and 650 nm emission (Synergy 4,
160 Biotek microplate reader, USA). Instrument calibration was achieved with standard solutions of
161 Tween-20 (0.01 to 0.05%). Data was expressed as relative fluorescence units (RFU)/mg proteins
162 in the homogenate.

163 *Data analysis*

164 The study design examines the bioavailability and toxicity in hydra exposed to the 7 increasing
165 concentrations of 50 and 100 nm NPs. The 96-h toxicity (50 % lethal concentration) was
166 determined using the Spearman-Kärber method and the 96-h sub-lethal effect concentration (50%
167 effect concentration) using the Log-logit method (Finney, 1964). Data normality and homogeneity
168 of variance were verified using the Shapiro-Wilk and Bartlett tests respectively. The data was
169 analysed using analysis of variance and critical differences between the controls and exposure
170 concentrations were determined using the Least Square Difference test. The trends between the
171 data were also analyzed using Pearson-moment correlation and principal component analysis. All
172 tests were performed using the SYSTAT software (version 13). Significance was set at $p < 0.05$.

173 **Results**

174 On a mass basis, the stock solutions of 50 nm polystyrene NPs (6.7×10^{14} particles/mL) contained
175 8 times more particles than the 100 nm NPs (8.3×10^{13} particles/mL). The size distribution of the
176 NPs were analyzed using dynamic light scattering and revealed that the NPs were distributed at
177 the specific diameter (50 and 100 nm) and did not form aggregates. The levels of NPs was
178 determined using the DCVJ probe methodology in hydra exposed for 96h to the NPs and following
179 a 24h depuration with the low concentrations (1.25-5 mg/L) and controls (Figure 2). The analysis
180 revealed that hydra accumulated both sizes of NPs in a concentration dependent manner. For the
181 50 nm NPs, the NPs were detected at the lowest concentration (1.25 mg/L) which persisted after a
182 24h depuration period. For the 100 nm, the NPs in hydra at the lowest concentration were more
183 abundant on a mass basis than the 50 nm NPs suggesting that the 100 nm NPs were more
184 bioavailable than 50 nm NP. Indeed, hydra accumulated 24 times more NPs with the 100 nm
185 diameter size even though they were 8 times less abundant than the 50 nm NPs. Although the
186 probe was shown to react with NPs in vitro, we could not determine if a small fraction was still
187 attached on the tentacles and in the gut although the hydra were washed. This is why a 24-
188 depuration period was done to determine if the NPs in Hydra could be removed in clean media
189 (where most of the NPs were still present at that time). The toxicity of the hydra was examined
190 based on the characteristic morphological changes (Figure 1). Although no mortality was observed
191 based on the complete regression/destruction of the hydra, the EC50 based on the shortened
192 morphology of the hydra was 3.6 (9.22×10^{11} particles/mL) and 18.4 mg/L (5.81×10^{11}
193 particles/mL) for the 50 and 100 nm NPs (Table 1). Hence, despite having a higher EC50, the 100
194 nm NPs were circa 1.7 times more toxic than the 50 nm on a particle basis. The biomass of hydra
195 was also significantly reduced at 14 (3.58×10^{12} particles/mL) and 28 mg/L (8.8×10^{11}

196 particles/mL) for the 50 and 100 nm NPs respectively, showing again that the 100 nm NPs
197 produced more toxicity than the 50 nm NPs on a particle amount basis.

198 The influence of NPs on the viscosity in hydra tissues was examined (Figure 3) and found to
199 increase in hydra extracts for both NPs sizes. For the 50 nm NPs, the viscosity increased in a
200 concentration dependent manner at concentrations > 2.5 mg/L. Viscosity persisted following the
201 24h depuration period with a significant increase at 2.5 mg/L. Correlation analysis revealed that
202 viscosity was significantly correlated with biomass ($r=-0.56$) and NPs in tissues ($r=0.60$) (Table
203 2). For the 100 nm, we obtained a peculiar bell-shaped response curve with an increase in viscosity
204 at low concentrations up to 2.5 mg/L NPs followed by a decrease at the higher concentrations. The
205 increased viscosity persisted after 24h depuration period with a significant increase at 5 mg/L
206 compared to controls. The formation of lipid-like LCs was examined using fluorescence
207 polarization spectroscopy (Figure 4). In hydra exposed to 50 nm NPs, a significant increase in
208 polarization was observed at the highest concentration tested (80 mg/L). There was no significant
209 increase in polarization after the 24h depuration period. In hydra exposed to the 100 nm NPs, a
210 significant increase in polarization was found at concentrations > 2.5 mg/L. Increased polarization
211 persisted after the 24h depuration period at 5 mg/L. Correlation analysis revealed that LCs
212 formation (polarisation) was significantly correlated with total lipids ($r= 0.40$) and NPs in tissues
213 ($r=0.46$) (Table 2). Changes in levels of polar lipids and LPO were also studied in hydra exposed
214 to the NPs (Figure 5A to 4D). In hydra exposed to 50 nm NPs, the levels of polar lipids were
215 significantly increased at concentrations between 5 and 80 mg/L (Figure 5A). The lipids levels
216 returned to control values after the 24h depuration period. The levels of LPO followed a biphasic
217 response where the levels were increased at low concentrations (1.25 to 5 mg/L) followed by a
218 decrease at higher concentrations where morphological effects were observed (Figure 5B). After

219 the 24h depuration period, LPO levels in controls were higher than the 96h control but the levels
220 returned to control values at 5 mg/L. In hydra exposed to the 100 nm NPs, the polar lipids levels
221 were significantly increased at concentrations ≥ 5 mg/L with the exception of the 40 mg/L owing
222 to data variability (Figure 5C). This effect persisted for the 5 mg/L treatment after the 24h
223 depuration period. Lipids levels were significantly correlated with viscosity ($r=0.59$) (Table 2).
224 LPO levels were significantly increased at concentrations ≥ 2.5 mg/L in respect to controls (Figure
225 5D). Oxidative stress persisted after the 24h depuration period with the 1.25 and 5 mg/L NP
226 concentration.

227 In the attempt to gain a global view on the effects of NPs on hydra, a principal component analysis
228 was performed on the 50 and 100 nm diameter NPs (Figure 6). In hydra exposed to the 50 nm NPs,
229 62 % of the total extracted variance was obtained with the biomarkers used in the present study
230 (Figure 6A). The most significant biomarkers were NPs levels in tissues (exposure), viscosity, LCs
231 and polar lipids (biomarkers of effects). Tissue levels of NP were closely related with lipids and
232 changes in viscosity. In hydra exposed to the more toxic 100 nm NPs, 60% of the total variance
233 was explained by the endpoints used in the study (Figure 6B), with NP, LCs formation, LPO and
234 viscosity changes being the most important biomarkers. NPs in tissues were closely related with
235 LPO, lipids levels and LC formation but weakly associated with viscosity. Lipids levels and the
236 formation of organized LCs were closely associated with each other which suggests that toxicity
237 involves the formation of organized (anisotropic) LCs in hydra exposed to NPs.

238 Discussion

239 The 100 nm diameter NP was 1.7 times more toxic than the 50 nm diameter NP on a particle
240 concentration basis. The EC50 for morphological changes was 3.6 and 18 mg/L for the 50 and 100
241 nm NPs respectively which was in the same range of the LC50 values (4.8 and 8.9 mg/L for 70

242 and 100 nm diameter NPs) for *Artemia salina* shrimp exposed to the polystyrene NPs in saline
243 waters (Mishra et al., 2019). In this study the smaller NPs showed more toxicity on a mass basis
244 than the larger ones as observed in *Hydra attenuata* but the equivalent amount of particles was not
245 provided. NPs produced oxidative stress as determined by LPO expression in hydra, which were
246 also induced in *Artemia*, corroborating our findings that polystyrene NPs produce oxidative
247 damage. Although oxidative stress (LPO) was involved in hydra exposed to the NPs, LPO levels
248 were more closely associated with levels of the 100 nm diameter NP than the 50 nm NP based on
249 principal component analysis. This could be explained by the observation of hydra losing their
250 tentacles at high concentrations of NPs potentially contributing to the variability in the levels of
251 internal NPs and oxidative stress. This is consistent with the peculiar bell-shaped response
252 obtained for LPO in hydra exposed to the 50 nm NPs. Oxidative stress was also observed in acs-
253 22 mutant *Caenorhabditis elegans* (nematodes) exposed to polystyrene NPs (Qu et al., 2018).
254 The NPs dysregulated some of the genes involved in the control of oxidative stress and activated
255 the expression of Nrf signaling which governs the antioxidant response pathway. In another study
256 with *C. elegans*, the uptake of 50 and 200 nm polystyrene NPs induced changes in metabolic
257 profiles and toxicity (Kim et al., 2019). The uptake of these NPs lead to perturbation in energy
258 metabolism (glucose and lactate) with induction of oxidative stress leading to reductions in
259 locomotion and reproduction. These observations are consistent with the present study were
260 reduction in tentacles and biomass were observed in hydra exposed to the NPs. In *Daphnia pulex*,
261 polystyrene NPs induced stress defence and immobilization during a 21-day exposure regime (Liu
262 et al., 2019). Indeed, as the fluorescent NP increased in the water fleas, genes involved in
263 antioxidant pathways (superoxide dismutase, catalase and glutathione S-transferase) initially
264 increased, followed by a decrease in gene expression. In zebrafish embryos, smaller-size

265 polystyrene NPs accumulated in lipid-rich regions such as in the egg yolk sac (Lee et al., 2019).
266 Oxidative stress and inflammation were also observed in zebrafish embryos exposed to the NPs.
267 This is consistent with the increase in polar lipids in hydra exposed to the NPs where the NPs in
268 tissues were significantly correlated with polar lipids, viscosity and the formation of organized
269 lipid-like LCs. This suggests that NPs could mobilize lipids into forming organized liquid crystal
270 and perhaps increasing oxidative damage in cells. The mobilization of lipids could also result from
271 the decreasing in hydra biomass (decrease in energy). For the 50 nm NPs, an analysis of covariance
272 of lipids levels with biomass as the covariate revealed that both the exposure concentration
273 ($p=0.002$) and biomass reduction ($p=0.02$) were significant, suggesting that both processes
274 occurred where the concentration of NPs was higher. In the case of 100 nm NPs, covariance
275 analysis revealed that the exposure concentration significantly influenced lipid levels with loss of
276 biomass having no significant effect. This suggests that lipids were mobilized in respect to NPs
277 exposure where a loss of biomass (fasting effect) had little influence on lipid contents in hydra.
278 The introduction of hydrophobic NPs such as polystyrene in cells could disrupt the conformational
279 changes of proteins and lipid organisation in the cytoplasm. For example in zebrafish embryos
280 exposed to NPs, the nanoparticles partitioned in lipid-rich environment of the yolk sac and induced
281 the expression of protein chaperones: heat shock proteins 70 and 90 (Kim et al., 2019).
282 Interestingly, the more toxic 100 nm NP revealed that NP in tissues were more closely associated
283 with organized lipid-like LCs and LPO in hydra. The FOE probe for LCs evaluation is composed
284 of an aliphatic carbon chain with a polar head (fluorescein) which can interact in hydrophobic
285 environments (lipid-like) which polarize fluorescence. Preliminary experiments revealed that NPs
286 alone do not significantly influence polarization but when combined with the post-mitochondrial
287 fraction, polarization occurs (Gagné et al., 2019a). It was proposed that fluorescence polarization

288 occurs at the lipid/protein corona of the NPs given the hydrophobic nature of the FOE probe. The
289 formation of lipid corona in addition to protein corona was also reported with other nanoparticles
290 such as colloidal gold (Zhang et al., 2018). Although the formation of lipid corona on gold
291 nanoparticles was governed by electrostatic interactions (cations at the surface proving better to
292 attract lipids than anionic charged nanoparticles), hydrophobic interactions could also induce
293 organized structures on the surface of polystyrene NPs (Gagné et al., 2019). In another study, oleic
294 acid-coated iron oxide nanoparticles were also toxic to neuronal cells (Fernandez-Bertolez et al.,
295 2018). Although the coated iron oxide nanoparticles were moderately toxic to cells, the compound
296 was able to produce single stranded DNA damage and reduced DNA repair activity in the presence
297 of H₂O₂ (oxidative stress). In human astrocytes, exposure to oleic acid-coated iron oxide
298 nanoparticles was also genotoxic, inducing primary DNA damage as determined by the comet
299 assay (Fernandez-Bertolez et al., 2019). However, the damage was seemingly reversible given that
300 no permanent cytogenetic damage (micronuclei) was observed. In conclusion, polystyrene NPs
301 were bioavailable to hydra and persisted after a 24 h depuration period. The 100 nm NPs was
302 more toxic than the 50 nm NP when compared on particle concentration basis. Toxicity was
303 dependent on the interaction between NP levels in hydra, lipids, the formation of organized LCs
304 and oxidative stress. The mobilization of lipids by the NPs could represent a mechanism for the
305 elimination of NPs from cells but could increase the toxicity (bioactivation) of NPs during this
306 process.

307
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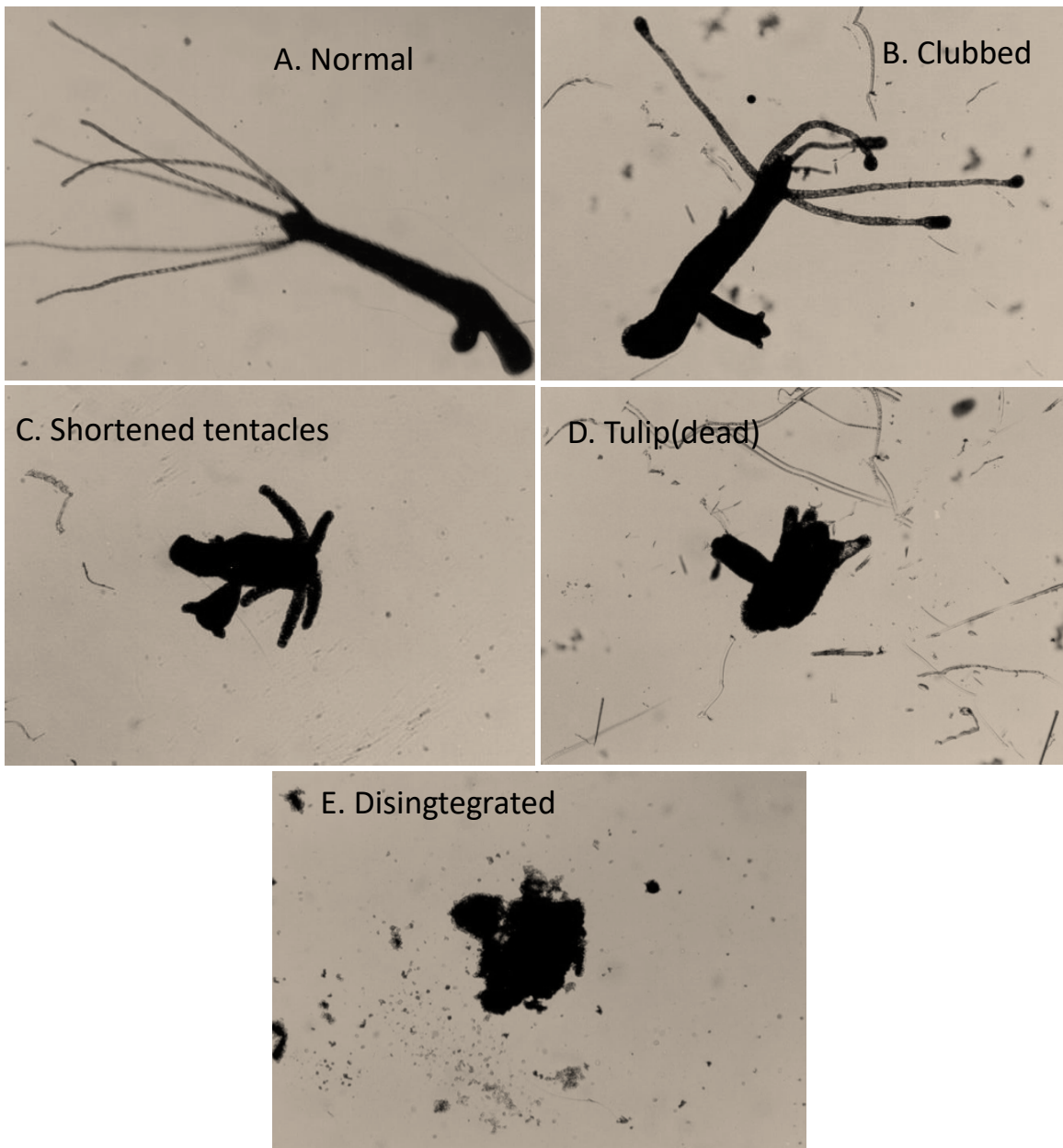
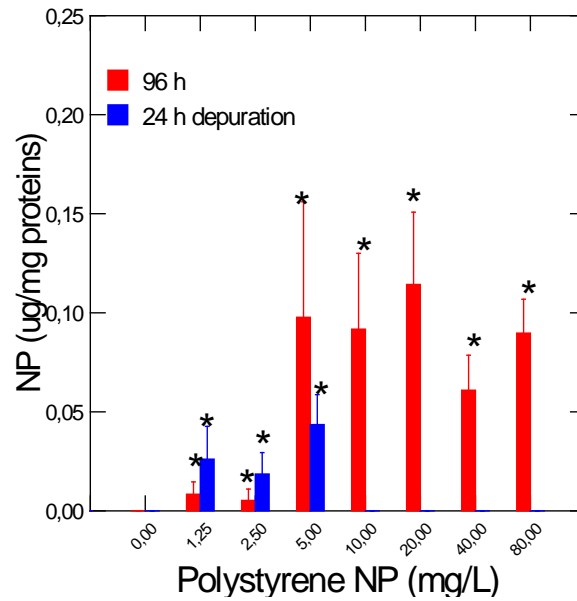


Figure 1. Morphological stages in *Hydra attenuate* after exposure to REEs for 96h (magnification x40): healthy polyp (A); clubbed tentacles (B); shortened tentacles(C); tulip stages (D) and disintegrated stage (E).

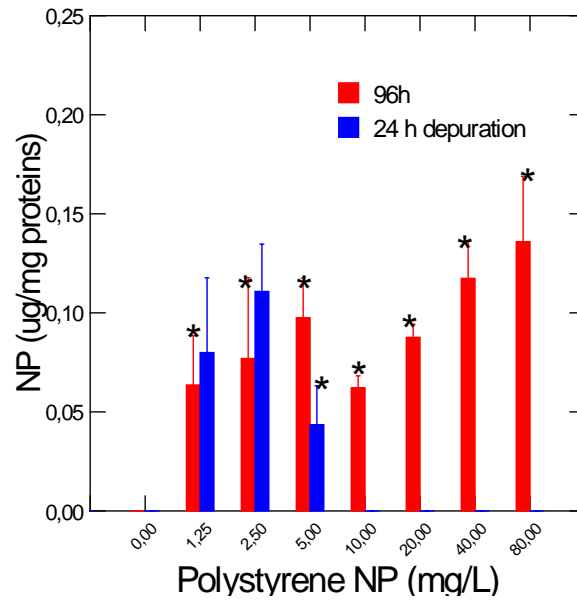
424 A 50 nm NP



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427 B 100 nm NP



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429 Figure 2. Detection of NP in *Hydra attenuata* using a molecular rotor probe.

430 Hydra were exposed to increasing concentrations of 50 nm (A) and 100 nm (B) polystyrene

431 nanoplastics for 96 h at 20°C. A subset of organisms were placed in clean media for 24 h for

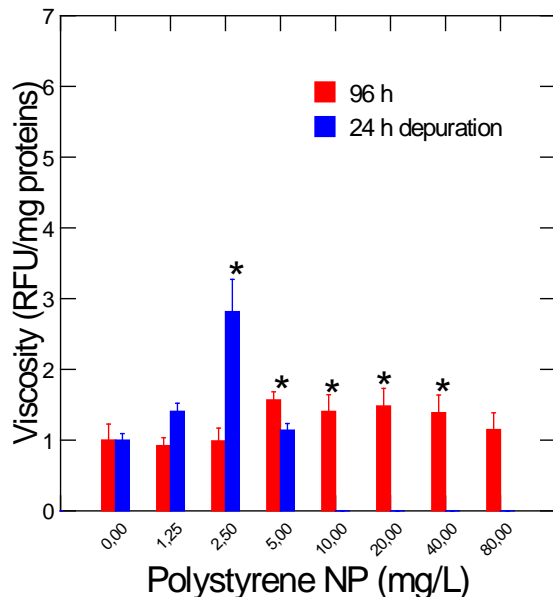
432 depuration. The data represent the mean with the standard error. The star * symbol indicated

433 significance from controls (0) at $p < 0.05$.

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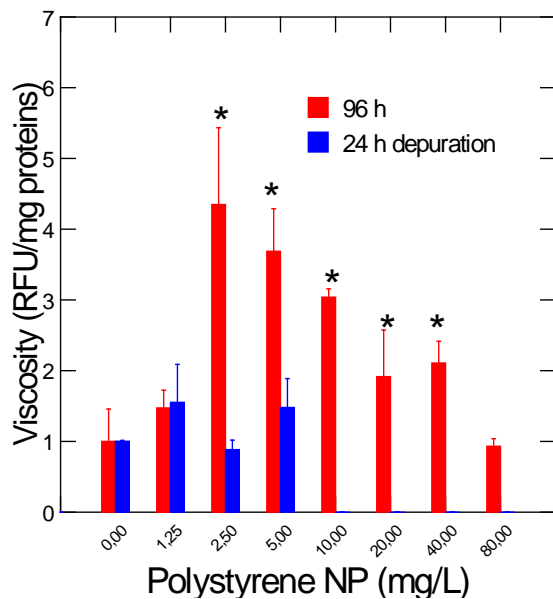
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436 A 50 nm NPs



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438 B 100 nm NPs



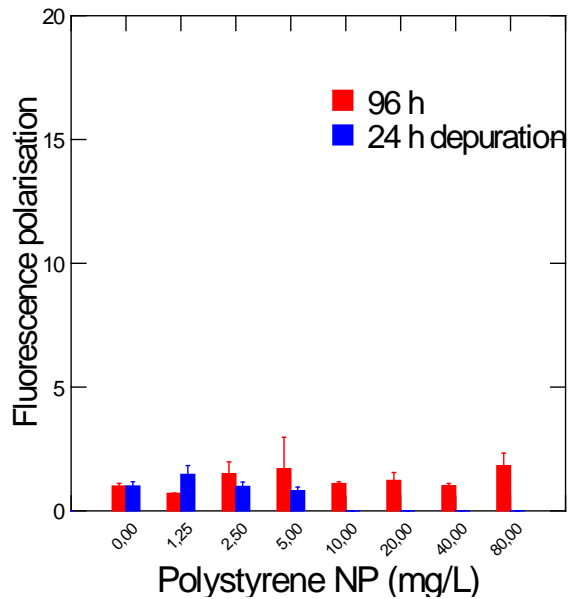
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440 Figure 3. Change in viscosity in *Hydra attenuata* tissues exposed to NPs
441 Hydra were exposed to increasing concentrations of 50 nm (A) and 100 nm (B) polystyrene
442 nanoplastics for 96 h at 20°C. A subset of organisms were placed in clean media for 24 h for
443 depuration. The data represent the mean with the standard error. The star * symbol indicated
444 significance from controls at $p < 0.05$.

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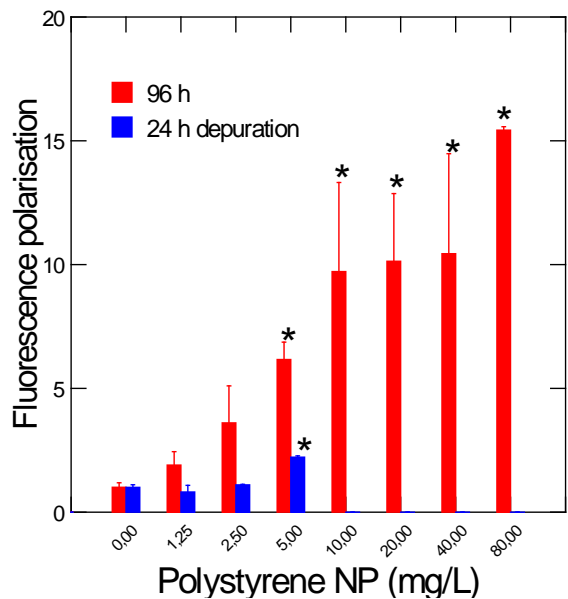
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447 A 50 nm NPs



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449 B 100 nm NPs



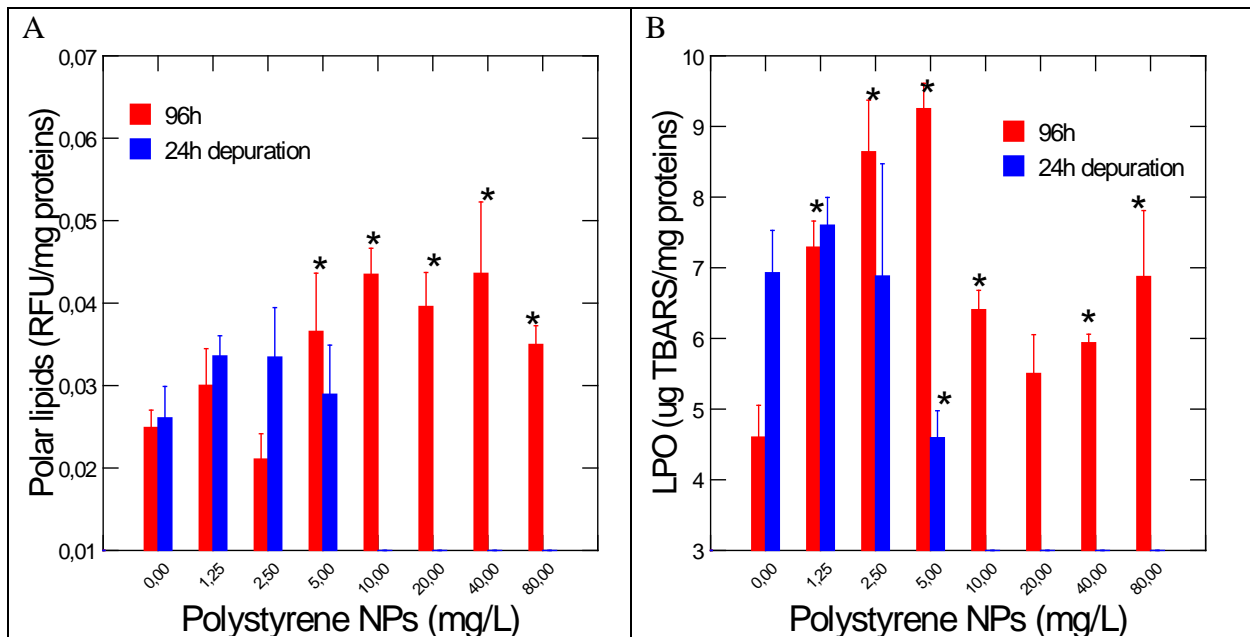
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451 Figure 4. Formation of lipid-like liquid crystals in *Hydra attenuata* exposed to NPs.
452 Hydra were exposed to increasing concentrations of 50 nm (A) and 100 nm (B) polystyrene
453 nanoplastics for 96 h at 20°C. A sub-set of organisms were placed in clean media for 24 h for
454 depuration. The data represent the mean with the standard error. The star * symbol indicated
455 significance from controls (0) at $p < 0.05$.

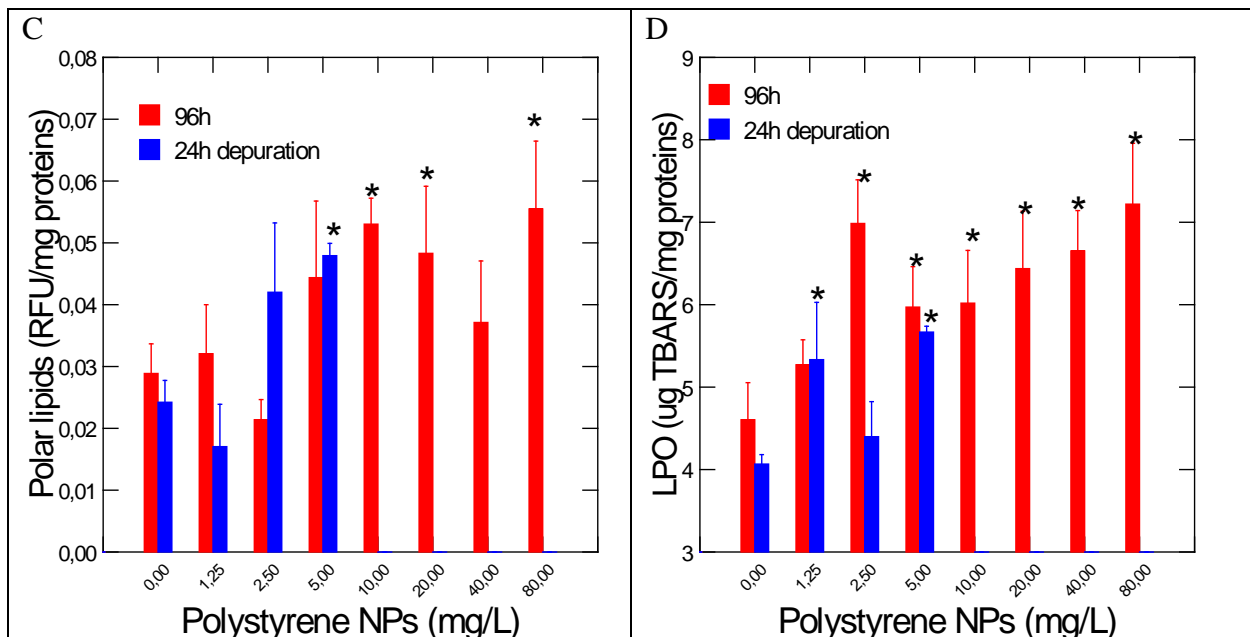
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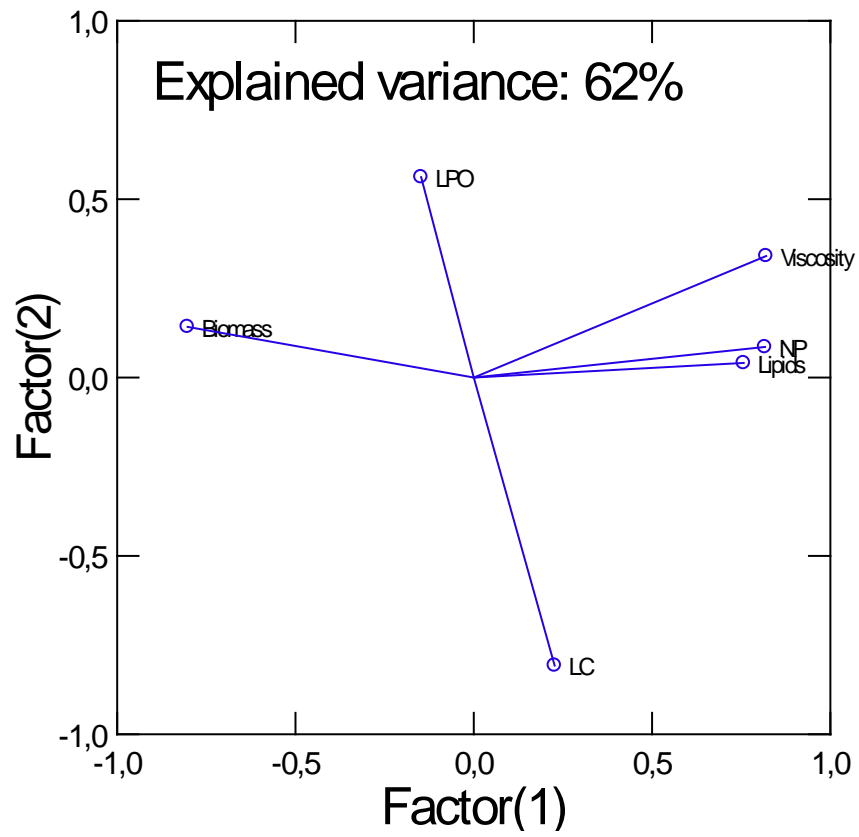
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463 Figure 5. Changes in lipid contents and oxidative stress in *Hydra attenuata* exposed to NPs

464 *Hydra* were exposed to increasing concentrations of 50 nm (A) and 100 nm (B) polystyrene
 465 nanoplastics for 96 h at 20°C. A subset of organisms were placed in clean media for 24 h for
 466 depuration. The data represent the mean with the standard error. The star * symbol indicated
 467 significance from controls (0) at $p < 0.05$.

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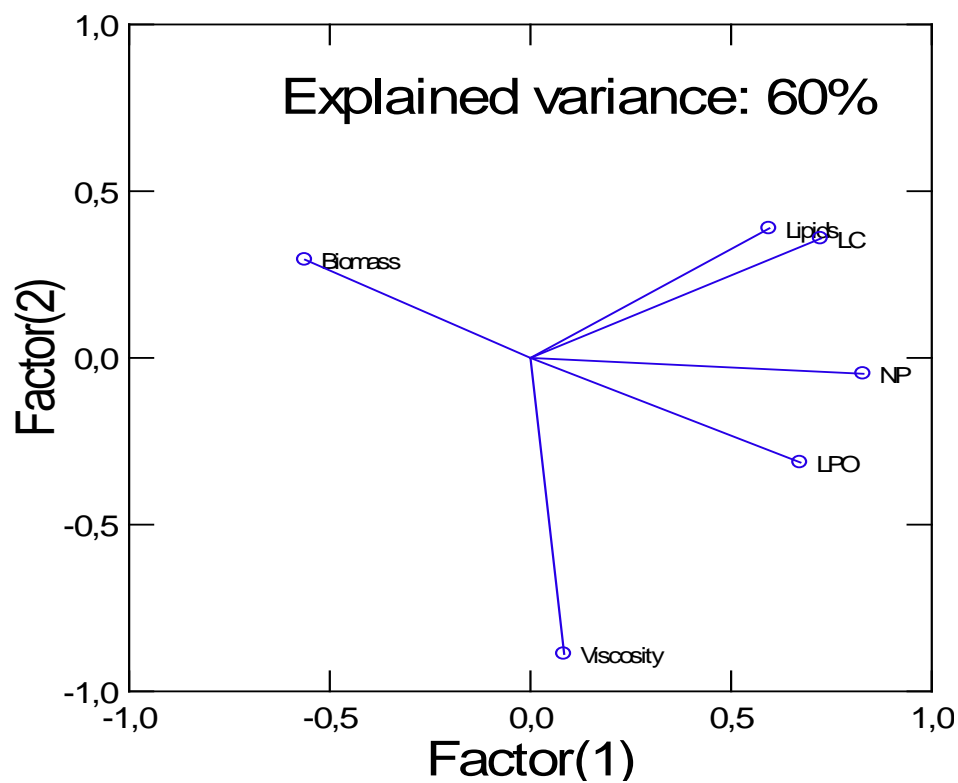
469 A 50 nm NPs



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472 100 nm NPs



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474 Figure 6. Factorial analysis of biomarker data in *Hydra attenuata* exposed to 50 nm (A) and 100
 475 nm (B) NPs. The biomarker responses were analyzed using principal component analysis.

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478 Table 1. Toxicity data of *Hydra attenuata* exposed to 50 and 100 nm polystyrene NPs

NP	LC50 in mg/L (95% confidence)	EC50 in mg/L (95% confidence)	Biomass ¹ (EC50)	Observation
50 nm	> 80 mg/L	3.6 (3-4.7)	14 mg/L	White deposits on antennae at >5 mg/L
100 nm	> 80 mg/L	18.4 (14-25)	28 mg/L	White deposits on antennae at >5 mg/L

479 1. Concentration that decrease the turbidity at 600 nm of hydra homogenates by 50 %.

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483 Table 2. Correlation analysis Table 1. Toxicity data of *Hydra attenuata* exposed to 50 and 100 nm
484 polystyrene NPs

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50 nm NP	Biomass	Lipids	NPs	Viscosity	LC	LPO
Biomass	1					
Lipids	-0.38	1				
NP	-0.59	0.49	1			
Viscosity	-0.56	0.59	0.60	1		
LC	-0.31	0.09	0.09	-0.04	1	
LPO	0.03	-0.20	-0.08	0.03	-0.08	1

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100 nm NP	Biomass	Lipids	NPs	Viscosity	LC	LPO
Biomass	1					
Lipids	-0.18	1				
NP	-0.44	0.33	1			
Viscosity	-0.18	-0.10	0.02	1		
LC	-0.23	0.40	0.46	-0.15	1	
LPO	-0.15	0.21	0.50	0.25	0.35	1

506

507 Significant correlations (p<0.05) are highlighted in **bold**. NP: Nanoplastics in hydra, LC: liquid
508 crystals (fluorescence polarisation), LPO: lipid peroxidation.