Polystyrene microplastics did not affect body growth and swimming activity in *Xenopus laevis* tadpoles

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21 # Abstract

22 A growing number of studies have highlighted the contamination and the effects towards organisms
23 of diverse microplastics (µPs) in the marine environment. Surprisingly, although the main sources of
24 µPs for marine environments are inland surface waters, the information on the occurrence and the
25 effects of µPs in freshwater ecosystems is still scant. Thus, the aim of the present work is to
26 investigate the ingestion and possible adverse effects due to the exposure to polystyrene µPs (PSµPs;
27 Ø = 3 µm) on tadpoles of the Amphibian *Xenopus laevis*. Larvae at the developmental stage 36, prior
28 to mouth opening, were exposed under semi-static conditions to 0.125, 1.25, and 12.5 µg/mL of
29 PSµPs, and allowed to develop until stage 46. At the end of the exposure, the digestive tract and the
30 gills from exposed and control tadpoles were microscopically examined, as well as changes in body
31 growth and swimming activity. PSµPs were observed in tadpoles' digestive tract, but not in the gills,
32 from each tested concentration. However, neither body growth nor swimming activity were affected
33 by PSµPs exposure. Our results demonstrated that PSµPs can be ingested by tadpoles, but they did
34 not alter *X. laevis* development and swimming behavior at least during early-life stages, also at high,
35 unrealistic concentrations.

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37 **Keywords:** Polystyrene microplastics; *Xenopus laevis*; ingestion; microscopy; swimming activity

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1. Introduction

Plastic contamination is a worrisome environmental problem gripping aquatic ecosystems worldwide. Over the past 50 years, an unfathomable amount of plastic debris has reached the marine environment, representing a serious hazard for seas and oceans at all latitudes (Thompson et al. 2004). Although the negative impact of big plastic debris (i.e. macroplastics; > 25 mm in size) on marine ecosystems has been highlighted since the '80s (Stefatos et al. 1999), a growing scientific interest has recently raised on microplastics. Microplastics (μPs) are small plastic particles (< 5 mm in size) that are produced *ex novo* to be used in cosmetics, industrial or medical applications, or derive from macroscopic debris after chemical, physical and biological breakdown (Barnes et al. 2009). A number of studies has identified marine ecosystems as hotspots of μPs pollution (Wright et al. 2013 and references therein), where they have been recorded up to a maximum estimated density of 100.000 particles/m^3 in surface waters and in the range of 100.000 items/ m^2 on shorelines (e.g. Desforges et al. 2014).

In spite of these findings, the contamination of freshwaters cannot be underestimated. In fact, freshwaters are the primary source of μPs entering seas and oceans through household sewage discharge (e.g., Fendall and Sewell 2009), direct input in water run-off or via storm-water and wastewater treatment plant outlets (Dris et al. 2015), spillage of plastic resin powders or pellets used for airblasting (Gregory 1996), and feedstocks used to manufacture plastic products (Zbyszewski et al. 2014) or, alternatively, from the breakdown of larger plastic items. Microplastics contamination of surface waters that has been reported was in the 0.001 - 0.1 items/m^2 range for lakes and 0.1 - 1 items/m^2 range for rivers, while in in the 10 – 10,000 items/m^2 and 1 – 1,000 items/m^2 for lake and river sediments, respectively (Dris et al. 2015). The presence of μPs in different environmental matrices and their small size can result in the ingestion by organisms. A wealth of studies has demonstrated the ingestion of different μP items in 160 marine species (see Lusher 2015 and reference therein), including fish (Collard et al. 2017), seabirds (Lavers et al. 2014), mammals (Fossi et al.

68 2012) and invertebrates (Graham et al. 2009; Cole et al. 2013; Messinetti et al. 2018), as well as in
69 39 freshwater species (Scherer et al. 2017). Experimental studies have also demonstrated that μPs
70 ingestion might negatively affect the health status of aquatic species, including fish (e.g. Lei et al.
71 2018), molluscs (e.g., Sussarellu et al. 2015) and crustacean (e.g., Frydkjaer et al. 2017). However,
72 such investigations have returned contrasting results mainly depending on μP size and shape, as well
73 as the tested concentration (Lee et al. 2013; Wright et al. 2013; Scherer et al. 2017).

74 Whilst evidence of strong negative effects, including intestinal damage, inhibition of feeding activity
75 and reduction of survival rates and body growth have been found (Lei et al. 2018; Murphy and Quinn
76 2018), some studies have pointed out slight or null adverse effects due to μPs ingestion (Hämer et al.
77 2014; Imhof et al. 2017; Weber et al. 2018). In spite of these findings, information on the impact of
78 μPs on swimming activity of aquatic organisms are still limited. However, this effect cannot be
79 neglected because ingestion of plastic microparticles could constrain organisms' movements in water.

80 To the best of our knowledge, only two studies have been focused on μPs ingestion on amphibian
81 species even though these organisms can be a target of μPs contamination, being exposed both in
82 aquatic and terrestrial ecosystems. Moreover, as amphibians are filter feeders until they complete
83 their metamorphosis, tadpoles are excellent models to investigate the ingestion of μPs and the
84 subsequent effects during early-life periods. A first laboratory study demonstrated the uptake,
85 accumulation and elimination of polystyrene μPs in *Xenopus tropicalis*, showing their presence in
86 both the digestive tract and on the gills (Hu et al. 2016). Similarly, a recent field work performed by
87 Hu and co-authors (2018) confirmed that tadpoles can ingest μPs from their surrounding environment,
88 showing the presence of different μPs typologies in the digestive tract of tadpoles belonging to four
89 different species sampled in small waterbodies of the Yangtze River Delta (China). Despite of these
90 findings, no study was focused on the potential adverse effects induced by μPs ingestion in tadpoles.
91 Thus, the present study was aimed at investigating the ingestion and the possible negative caused by
92 polystyrene spherical microplastics (PμPs; Ø = 3 μm) on *Xenopus laevis* tadpoles. We exposed *X.*

93 *laevis* tadpoles to three increasing concentration of PSμPs (0.125; 1.25 and 12.5 μg mL^{-1}) from stage
94 36, prior to mouth opening, to stage 46 (Nieuwkoop and Faber 1994). At the end of the exposure we
95 assessed the ingestion of PSμPs in tadpoles' digestive tract and gills, as well the effects on survival,
96 body growth and swimming activity.

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98 ## 2. Materials and Methods

99 *2.1 Chemicals and Polystyrene microplastic preparation*

100 All analytical grade reagents, L-cysteine, 3-amino-benzoic acid ethyl ester (MS222), salts for FETAX
101 solution, and blue polystyrene microplastics (PSμPs; Ø=3 μm) were purchased from Sigma-Aldrich,
102 Milano, Italy. Chemical-physical properties of the μP beads were tested. The size of polystyrene μPs
103 was assessed by measuring size of 500 particles on different pictures captured with a Scanning
104 Electron Microscope (SEM) (Figure S1) using Fiji freeware software (Schindelin et al. 2012),
105 resulting in 2.75 ± 0.09 μm of diameter. Polystyrene μPs were chemically characterized by using a
106 Fourier Transformed Infrared Spectroscope (FT-IR) Perkin Elmer Spectrum 100: PSμPs were
107 analyzed as received. Subsequently 10 mL of FETAX solution was dried at room temperature
108 overnight (16 hours) together with the same volume of a FETAX solution containing the PSμP (50
109 μg mL^{-1}). The two residues were compared with the PSμPs. In Figure 1 the spectra obtained are
110 overlapped and signals showing the presence of PSμP are indicated. We focused on PSμPs because
111 this polymer is one of the most abundant in both marine and freshwater ecosystems (Li et al. 2016).
112 Moreover, polystyrene has a negligible styrene release in water solution, therefore we can be
113 reasonably sure that possible effects are due to the physical presence of μPs and not to monomer
114 release (Cohen et al. 2002). The commercial standard was an aqueous suspension (50 mg/mL) that
115 was diluted in culture medium to obtain a stock solution of 50 μg/mL concentration. Three PSμPs
116 concentration, namely 0.125 (1×10^5 particles mL^{-1}), 1.25 (2.833×10^5 particles mL^{-1}) and 12.5

117 µg/mL (8.666 x 10^5 particles mL^{-1}) were tested according to previous works on other aquatic
118 organisms (Lee et al. 2013; Messinetti et al. 2018).

119 *2.2 Animals and experimental design*

120 Adults of *Xenopus laevis* were maintained at the University of Milan in aquaria filled with
121 dechlorinated tap water at 22 ± 2 °C, with a 12 h light/dark cycle and fed a semi-synthetic diet
122 (Mucedola S.r.L., Settimo Milanese, Italy). Embryos were obtained from natural breeding of adult
123 pairs and the experiment run according to the Frog Embryo Teratogenesis Assay-Xenopus, FETAX,
124 protocol (ASTM 1998), lightly modified. In particular, we planned a late exposure, being interested
125 in the possible effects of ingested PSµPs and not to their developmental toxicity. Embryos were thus
126 exposed prior to mouth opening, which happens at stage 40 (Nieuwkoop and Faber 1994), and not at
127 the classic midblastula stage (stage 8). At the end of the test, (stage 46), FETAX endpoints i.e.
128 mortality and growth inhibition were considered. Exposure tests were performed in FETAX solution
129 (0.01 M NaCl, 1 mM $NaHCO_3$, 0.4 mM KCl, 0.1 mM $CaCl_2$, 0.35 mM $CaSO_4$ and $2H_2O$, and 0.6
130 mM $MgSO_4$**,** at pH 7.6–8.0).
131 After breeding, adults were removed and embryos collected in plastic Petri dishes. Fertilized eggs
132 were dejelled with 2% L-cysteine solution (pH 8.0) and rinsed several times with FETAX solution..
133 Normally cleaved embryos were selected, transferred to plastic Petri dishes filled with 10 mL of
134 FETAX solution and allowed to develop until stage 36-37, according to Nieuwkoop and Faber (1994).
135 Thirty tadpoles at stage 36-37 were seeded in Petri dishes and exposed to a nominal concentration of
136 0.125, 1.25 and 12.5 µg mL^{-1} PSµPs in FETAX. The test was performed in semi-static conditions
137 every single day. All groups were incubated in a thermostatic chamber at 22 ± 0.5 °C, and both control
138 and PSµPs exposure groups duplicated. Tadpoles were not fed during the experiment and allowed to
139 develop until stage 46, end of the exposure test. At this point, 20 tadpoles from each group were
140 transferred to a small Petri dish filled with 5 mL of culture medium to be video-tracked. Then, all
141 tadpoles were anaesthetized with MS222 at a final concentration of 100 mg L^{-1} and evaluated for

142 single malformations under a dissecting microscope. At the end of the analysis, all samples were fixed
143 in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer solution (pH 7.4) for growth retardation
144 measurements and for the subsequent microscopical analyses.
145
146 *2.4 Microscopy analyses*
147 For light microscopy analyses, 26 tadpoles per replicate were dehydrated in Ethanol (EtOH) up to
148 70% and examined under a Leica DMRA2 microscope. Images were collected with a Leica DC300F
149 digital camera and tadpole body lengths measured using Fiji freeware software (Schindelin et al.
150 2012). For electron microscopy analyses, 10 tadpoles from each treatment group were randomly
151 selected, post-fixed in 1% OsO_4 for 2 hours at 4 °C and critical-point dried in a Balzers Unions CPD
152 020 apparatus (Balzers Unions, Lichtenstein). Under a stereomicroscope, the digestive tract and gills
153 of each tadpole were dissected, mounted onto standard SEM stubs, gold sputtered, and observed under
154 a Zeiss LEO 1430 SEM at 20 kV.
155
156 *2.5 Swimming activity analysis*
157 The effects on swimming activity of tadpoles were evaluated by a video tracking analysis. Twenty
158 tadpoles per treatment, including control, were randomly selected from exposure Petri dishes and
159 individually transferred to another Petri dish (Ø = 60 mm) filled with 5 mL of culture medium.
160 Because of their high motility, tadpoles were enclosed in a small arena (Ø = 10 mm) placed in the
161 center of the Petri dish where they stopped movements and acclimatized for 3 minutes to new
162 conditions. After acclimatization, the small arena was removed, the tadpoles restarted to swim and its
163 movements were filmed by an iPhone 6 for 10 seconds. The obtained 1080p Full HD videos were
164 analyzed by using the ImageJ plugin Animal Track (Gulyàs et al. 2016). The distance moved (mm)
165 and mean swimming speed (cm/sec) were considered as swimming activity endpoints.
166

167 *2.6 Statistical analysis*

168 The effect of PSμPs exposure on the body length and the swimming activity of tadpoles was
169 investigated by using linear mixed models (LMMs) including the treatment as fixed factor, while the
170 identity of the Petri dish as a random factor. As no mortality occurred in all the experimental groups
171 no statistical analysis on this endpoint was performed. The analyses were run using SPSS 21.0
172 statistical package.

173

174 **3. Results and discussion**

175 The present work showed that *X. laevis* tadpoles can ingest polystyrene microplastic beads during
176 early-life stages, even though these particles did not significantly affect survival, body growth and
177 swimming activity. Stereomicroscopy analyses showed the presence of PSμPs in the whole digestive
178 tract of tadpoles, mostly after the exposure to 1.25 μg mL^{-1} and 12.5 μg mL^{-1}, while the lower
179 concentration seems not to show the presence of PSμPs (Figure 2). However, the SEM analyses
180 showed the presence of PSμPs in the digestive tract of tadpoles from all the treatments, including
181 0.125 μg mL^{-1} (Figure 3). As expected, the digestive tract from tadpoles exposed to 12.5 μg mL^{-1} of
182 PSμPs was completely full of particles, while the amount of microbeads was notably lower in the
183 individuals from the other treatments. SEM analyses suggest the absence of mechanical damage to
184 the walls of the epithelium. Our findings are in agreement with previous studies demonstrating that
185 polystyrene μPs of different size can be easily ingested and accumulated in the digestive tract of
186 different aquatic organisms. For instance, PSμPs (1.7 - 30.6 μm in size) were observed in the digestive
187 tract of thirteen marine zooplanktonic organisms (Cole et al., 2013), while 100 nm – 10 μm PSμPs
188 filled up the digestive tract of the cladoceran *Daphnia magna* (Ma et al. 2016; Rist et al. 2017).
189 Moreover, similar results were also obtained in *Xenopus tropicalis*, whereby fluorescent polystyrene
190 μPs (1 and 10 μm in size) were clearly observed in alimentary canal, stomach and intestine of tadpoles

191 already after 1 h of exposure (Hu et al. 2016). However, in our study no PSµPs were found on tadpole
192 gills at each tested concentration (Figure S2), contrasting previous results on *X. tropicalis* that showed
193 the presence of 1 and10 µm on the gills of tadpoles (Hu et al. 2016). Similarly, 8 - 10 µm PSµPs were
194 found on the gills of the crab *Carcinus maenas* (Watts et al. 2014). Such findings suggested that the
195 ingestion, the transfer and the accumulation of different µPs in specific body districts greatly depends
196 on the concentration and the size of the particles, as well as on the size of the focal model species
197 (Wright et al. 2013). Thus, we suppose that the discrepancy in the presence of µPs on the gills of two
198 *Xenopus* species might be due to their different body size. In fact, *X. tropicalis* is smaller than *X.*
199 *laevis*, and consequently it owns smaller gills and thick filaments, which allowed a more efficient
200 trapping of PSµPs. This anatomic feature could also explain the higher accumulation of PSµPs in *X.*
201 *tropicalis* compared to *X. laevis* although the exposure concentration selected by Hu and co-authors
202 (2016) were notably lower (concentration range of 1 µm PSµPs: $10\text{-}10^5$ particles mL^{-1} and
203 concentration range of 10 µm PSµPs: $0.1\text{-}10^3$ particles mL^{-1}) than those tested in our study
204 (concentration range of 3 µm PSµPs: 1×10^5 - 8.6×10^5 particles mL^{-1}.
205 Although PSµPs filled up the digestive tract of tadpoles, none tadpole died over the exposure period
206 neither in the control nor in all the treatment groups. Our results are consistent with previous studies
207 showing no mortality on diverse aquatic organisms after the exposure to diverse concentrations of
208 dissimilar µP polymers, including invertebrates (e.g., Imhof et al., 2017; Rist et al., 2017; Weber et
209 al., 2018) and vertebrates (e.g., Hu et al., 2016; Chen et al., 2017). However, the ingestion of PSµPs
210 might cause sub-lethal effects, including the reduction of food assimilation and body growth (Cole et
211 al. 2015; Xu et al. 2017). No significant differences in body length of tadpoles at stage 46 was noted
212 between the treatment groups and the control ($F_{3,203} = 1.137$; $P = 0.335$; Figure 4), suggesting that
213 PSµPs ingestion did not affected body growth of tadpoles during early-life stages. Our results are in
214 contrast with previous studies demonstrating that the ingestion of PSµPs negatively affected body
215 growth of diverse organisms (Besseling et al. 2014; Lo et al. 2018). These discrepancies might be due
216 to the duration of the exposure and/or the size of the tested µPs. In fact, 14-day exposure to PSµPs

217 (Ø = 2 - 2.4 µm) reduced the growth of the onyx slipper snail *Crepidula onyx* (Lo et al. 2018).
218 Moreover, the 21-day exposure to polystyrene nanoplastics (Ø = 70 nm) reduced the growth of
219 *Daphnia magna* (Besseling et al. 2014), while the exposure up to 7 days post-fertilization to
220 polystyrene nanoplastics (Ø = 50 nm) altered the early development of *X. laevis* (Tussellino et al.
221 2015). We may suppose that ingested PSµPs did not affect body growth of *X. laevis* tadpoles because
222 they do not interfere with the assimilation of yolk reserves used during early-life stages. Alternatively,
223 polystyrene microbeads were ingested and egested quickly by tadpoles (Hu et al. 2016) and did not
224 affect the development.
225 Despite no developmental effects, the ingestion of µPs could affect tadpole swimming activity
226 because particles can represent an additional weight for tadpoles and consequently a high energy
227 demanding effort to be supported. According to results on body growth, PSµPs ingestion did not
228 affect the swimming activity of tadpoles (Figure 5 a-b); no significant differences in terms of distance
229 moved ($F_{3,73} = 0.677$; $P = 0.569$) and mean swimming speed ($F_{3,73} = 0.196$; $P = 0.899$) occurred
230 between the treatment groups and the control. On the contrary a previous study of the amphipod
231 *Platorchestia smithi* showed that the ingestion of polyethylene µPs (Ø = 35-45 µm) caused a decrease
232 of the jump height (Tosetto et al. 2016). This discrepancy can be due to species-specific differences,
233 different ontogenetic stage and/or to the type of analyzed swimming activity of the model organisms.
234 In fact, in the present study we monitored the horizontal swimming of tadpoles, while Tosetto and
235 co-authors (2016) monitored the vertical hopping of amphipods. In addition, the rate of µP
236 ingestion/egestion, the size and the composition of plastic used for exposures (3 µm polystyrene used
237 in our study versus 35-45 µm polyethylene particles used by Tosetto et al., 2016), and their exposure
238 concentration can affect the swimming activity and explain the differences of the responses after µP
239 exposure. Lastly, Tosetto and co-author (2016) 'doped' the polyethylene µPs administered to
240 amphipods with contaminated marine water and doped µPs adsorbed on their surface 0.007 $\mu g\ g^{-1}$ of
241 PAHs, which could cause the observed behavioral changes. This hypothesis is supported by a
242 previous study of zebrafish larvae showing that negative effects on swimming activity occurred only

243 when organisms where co-exposed to μPs and α-ethynylestradiol, while no swimming alteration was
244 noted when larvae were exposed to μPs alone (Chen et al. 2017).

245

246 4. Conclusion

247 Our findings showed that 3 μm PSμPs are quickly ingested by *X. laevis* tadpoles at all the tested
248 concentrations, but the exposure period does not induce negative effects on the body growth and
249 swimming activity, also at high unrealistic concentrations. Further studies should be planned in order
250 to evaluate if long-term exposure can impact the development and post-metamorphic stages of *X.*
251 *laevis*. Lastly, investigations on the potential effects due to smaller polystyrene spherical particles or
252 to fragments, foams and pellets, which are predominant in freshwater ecosystems, should be
253 necessary to understand the real impact of PSμPs on aquatic organisms.

254

255 5. References

256 Barnes DKA, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic
257 debris in global environments. Phil Trans R Soc B 364: 1985-1998.
258 Besseling E, Wang B., Lürling M, Koelmans AA (2014) Nanoplastic Affects Growth of *S. obliquus*
259 and Reproduction of *D. magna*. Environ Sci Techn 48:12336-12343.
260 Chen Q, Gundlach M, Yang S, Jiang J, Velki M, Yin D, Hollert H (2017) Quantitative investigation
261 of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity.
262 Sci Total Environ 584:1022-1031.
263 Cohen JT, Carlson G, Charnley G, Coggon D, Delzell E, Graham JD, Greim H, Krewski D, Medinsky
264 M, Monson R (2002) Comprehensive Evaluation of the Potential Health Risks Associated with
265 Occupational and Environmental Exposure to Styrene. J Toxicol Environ Health B 5:1– 263.

266 Cole M, Lindeque P, Fileman E, Halsband C, Goodhead R, Moger J, Galloway TS (2013)
267 Microplastic ingestion by zooplankton. Environ Sci Techn 47:6646-6655.
268 Cole M, Lindeque P, Fileman E, Halsband C, Galloway TS (2015) The Impact of Polystyrene
269 Microplastics on Feeding, Function and Fecundity in the Marine Copepod *Calanus*
270 *helgolandicus*. Environ Sci Techn 49:1130-1137.
271 Collard F, Gilbert B, Compère P, Eppe G, Das K, Jauniax T, Parmentier E (2017) Microplastics in
272 livers of European anchovies (*Engraulis encrasicolus*, L.). Envrion Poll 229:1000-1005.
273 Desforges JW, Galbraith M, Dangerfield N, Ross PS (2014) Widespread distribution of microplastics
274 in subsurface seawater in the NE Pacific Ocean. Mar Poll Bull 15:94-99.
275 Dris R, Imhof H, Sanchez W, Gasperi J, Galgani F, Tassin B, Laforsch C (2015) Beyond the ocean:
276 contamination of freshwater ecosystems with (micro-)plastic particles. EnvironChem 12:539-
277 550.
278 Fendall LS, Sewell MA (2009) Contributing to marine pollution by washing your face: Microplastics
279 in facial cleanser. Mar Poll Bull 58: 1225-1228.
280 Fossi MC, Panti C, Guerranti C, Coppola D, Giannetti M, Marsili L, Minutoli R (2012) Are baleen
281 whales exposed to the threat of microplastic? A case study of the Mediterranean fin whale
282 (*Balaenoptera physalus*). Mar Poll Bull 64:2374-2379.
283 Frydkjaer C, Iversen N, Roslev P (2017) Ingestion and Egestion of Microplastics by the Cladoceran
284 *Daphnia magna*: Effects of Regular and Irregular Shaped Plastic and Sorbed Phenanthrene. Bull
285 Environ Contam Toxicol 99:655–661.
286 Graham ER, Thompson JT (2009) Deposit- and suspension-feeding sea cucumbers (Echinodermata)
287 ingest plastic fragments. J Exp Mar Bio Ecol 368:22-29.
288 Gregory MR (1996) Plastic “scrubbers” in hand cleansers: a further (and minor) source for marine
289 pollution identified. Mar Poll Bull 32:867-871.

290 Gulyás M, Bencsik N, Pusztai S, Liliom H, Schlett K (2016) Animal Tracker: An ImageJ-Based
291 Tracking API to Create a Customized Behaviour Analyser Program. Neuroinformatics, Epub
292 DOI 10.1007/s12021-016-9303-z.
293 Hämer J, Gutow L, Köhler A, Saborowski R (2014) Fate of Microplastics in the marine isopod *Idotea*
294 *emarginata*. Environ Sci Techn 48:13451-13458.
295 Hu L, Su L, Xue Y, Mu J, Xu J, Shi H (2016) Uptake, accumulation and elimination of polystyrene
296 microspheres in tadpoles of *Xenopus tropicalis*. Chemosphere 164:611-617.
297 Hu L, Chernick M, Hinton DE, Shi H (2018) Microplastics in Small Waterbodies and Tadpoles from
298 Yangtze River Delta, China. Environ Sci Techn 52:8885-8893.
299 Imhof HK, Rusek J, Thiel M, Wolinska J, Laforsche C (2017) Do microplastic particles affect
300 Daphnia magna at the morphological, life history and molecular level? PlosOne DOI:
301 10.5061/dryad.9d84.
302 Lavers JL, Bond AL, Hutton I (2014) Plastic ingestion by Flesh-footed Shearwaters (*Puffinus*
303 *carneipes*): Implications for fledgling body condition and the accumulation of plastic-derived
304 chemicals. Environ Poll 187:124-129.
305 Lee K, Shim WJ, Kwon O, Kang J (2013) Size-Dependent Effects of Micro Polystyrene Particles in
306 the Marine Copepod *Tigriopus japonicus*. Environ Sci Techn 47:11278 – 11283.
307 Lei L, Wu S, Lu S, Liu M, Song Y, Fu Z, Shi H, Raley-Susman K M, He D (2018) Microplastic
308 particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode
309 *Caenorhabditis elegans*. Sci Tot Envir 619:1-8.
310 Li WC, Tse HF, Fok L (2016) Plastic waste in the marine environment: A review of sources,
311 occurrence and effects. Sci Tot Environ 566:333-349.
312 Lo HKA, Chan KYK (2018) Negative effects of microplastic exposure on growth and development
313 of *Crepidula onyx*. Environ Poll 233:588-595.
314 Lusher A (2015) Microplastics in the Marine Environment: Distribution, Interactions and Effects.
315 Marine Anthropogenic Litter 245:307.

316 Ma Y, Huang A, Cao S, Sun F, Wang L, Guo H, Ji R (2016) Effects of nanoplastics and microplastics
317 on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. Environ
318 Poll 219:166-173.
319 Messinetti S, Mercurio S, Parolini M, Sugni M, Pennati R, (2018) Effects of polystyrene microplastics
320 on early stages of two marine invertebrates with different feeding strategies. Environ Poll
321 237:1080-1087.
322 Murphy F and Quinn B (2018) The effects of microplastic on freshwater *Hydra attenuate* feeding,
323 morphology & reproduction. Environ Poll 234:487-494.
324 Nieuwkoop PD, Faber J, (1994). Normal table of *Xenopus laevis* (Daudin): a systematical and
325 chronological survey of the development from the fertilized egg till the end of metamorphosis.
326 Rist S, Baun A, Hartmann N (2017) Ingestion of micro- and nanoplastics in *Daphnia magna* -
327 Quantification of body burdens and assessment of feeding rates and reproduction. Environ Poll
328 228:398-407.
329 Scherer C, Brennholt N, Reifferscheid G, Wagner M (2017) Feeding type and development drive the
330 ingestion of microplastics by freshwater invertebrates. Sci Rep 7.
331 Schindelin J; Arganda-Carreras I, Frise E (2012) Fiji: an open-source platform for biological-image
332 analysis. Nature methods 9: 676-682
333 Stefatos A, Charalampakis M, Papatheodorou G, Fereninos G (1999) Marine Debris on the Seafloor
334 of the Mediterranean Sea: Examples from Two Enclosed Gulfs in Western Greece. Mar Poll Bull
335 38: 389-393.
336 Sussarellu R, Suquet M, Thomas Y, Lambert C, Fabioux C, Pernet MEJ, Le Goïc N, Quillien V,
337 Mingant C, Epelboin Y, Corporeau C, Guyomarch J, Robbens J, Paul-Pont I, Soudant P, Huvet
338 A (2015) Oyster reproduction is affected by exposure to polystyrene microplastics. Proc Natl
339 Acad Sci USA 113:2430-2435.
340 Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG, McGonigle D, Russell AE
341 (2004) Lost at Sea: Where is all the plastic? Science 304: 838.

342 Tosetto L, Broen C, Williamson J (2016) Microplastics on beaches: ingestion and behavioural
343 consequences for beachhoppers. Mar Biol 163:199.
344 Tussellino M, Ronca R, Formiggini F, De Marco N, Fusco S, Netti PA, Carotenuto R (2015)
345 Polystyrene nanoparticles affect *Xenopus laevis* development. J Nanops 17:70.
346 Watts AJR, Lewis C, Goodhead RM, Beckett SJ, Moger J, Tyler CR, Gallowat TS (2014) Uptake
347 and Retention of Microplastics by the Shore Crab *Carcinus maenas*. Environ Sci Techn48: 8823–
348 8830.
349 Weber A, Scherer C, Brennholt N, Reifferscheid G, Wagner M (2018) PET microplastics do not
350 negatively affect the survival, development, metabolism and feeding activity of the freshwater
351 invertebrate *Gammarus pulex*. Environ Poll 234:181 - 189.
352 Wright SL, Thompson RC, Galloway TS (2013) The physical impacts of microplastics on marine
353 organisms: A review. Environ Poll 178483 - 492.
354 Xu XY, Lee WT, Chan AKY, Lo HS, Shin PKS, Cheung SG (2017) Microplastic ingestion reduces
355 energy intake in the clam *Atactodea striata*. Mar Poll Bull 124: 798 – 802.
356 Zbyszewski M, Corcoran PL, Hockin A (2014) Comparison of the distribution and degradation of
357 plastic debris along shorelines of the Great Lakes, North America. J Great Lakes Res 40: 288-
358 299.
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368 # Figure captions

369 Figure 1: chemical characterization of blue PSμPs by a Fourier Transformed Infrared Spectroscope
370 (FT-IR). Spectra of PSμPs (blue), FETAX solution (red) and FETAX solution containing PSμPs
371 (black) are reported. Black arrows indicate the specific peaks of polystyrene.

372

373 Figure 2. Ventral view of *X. laevis* tadpoles (stage 46) at the steromicroscope. (A) Control sample;
374 (B) Sample exposed to 0.125 μg/mL PSμPs showing no sign of blue beads in the digestive
375 system; (C) and (D) Samples exposed to 1.25 (C) and 12.5 μg/mL (D) showing large amounts of
376 PSμPs in their gut. Scale bar = 1 mm

377

378 Figure 3. SEM images from the digestive epithelium of *X. laevis* tadpoles showing the increasing
379 presence of PSμPs into the lumen. LM = Low Magnification; HM = High Magnification; Black
380 arrowhead = Brush border; * = Intestinal wall.

381

382 Figure 4. Estimated marginal means (± standard error) of total body length of *X. laevis* tadpoles (stage
383 46). Letters above histograms indicate differences between groups, whereby similar letters
384 indicate no significant differences. . No significant differences were found ($p > 0.05$).

385 .

386 Figure 5. Estimated marginal means (± standard error) of distance moved (a) and swimming speed
387 (b) measured in *X. laevis* tadpoles (stage 46). Letters above histograms indicate differences
388 between groups, whereby similar letters indicate no significant differences. No significant
389 differences were found ($p > 0.05$).

390

391 Figure S1. SEM images showing the 3 μm PSμPs used for the exposures.

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393 Figure S2. SEM images showing the structure of gills from *X. laevis* tadpoles at stage 46. (A) Control
394 sample; (B) Sample exposed to 12.5 μg/mL PSμPs. No polystyrene beads were found.

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