Science of the Total Environment 631–632 (2018) 421–428

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Transgenerational effects and recovery of microplastics exposure in model populations of the freshwater cladoceran *Daphnia magna* Straus

Alexandra Martins, Lúcia Guilhermino *

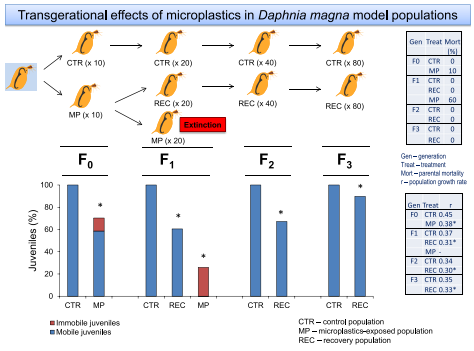
ICBAS – Institute of Biomedical Sciences of the University of Porto, Department of Populations Study, Laboratory of Ecotoxicology (ECOTOX), Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

CIIMAR – Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, Research Team of Ecotoxicology, Stress Ecology and Environmental Health (ECOTOX), Avenida General Norton de Matos, 4450-208 Matosinhos, Portugal

HIGHLIGHTS

- Transgerational effects and recovery from microplastics exposure were investigated in *D. magna*
- Microplastics (0.1 mg/l) decreased growth, reproduction and population growth rate
- Microplastics caused the extinction of microplastics-exposed population in 2 generations
- The recovery model population did not recover completely up to the F_3 generation

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 31 January 2018
Received in revised form 4 March 2018
Accepted 5 March 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Microplastics
Daphnia magna
Transgenerational study
Developmental and reproductive toxicity
Human health warning

ABSTRACT

The environmental contamination by microplastics is a global challenge to ecosystem and human health, and the knowledge on the long-term effects of such particles is limited. Thus, the effects of microplastics and post-exposure recovery were investigated over 4 generations (F_0, F_1, F_2, F_3) using *Daphnia magna* as model. Effect criteria were parental mortality, growth, several reproductive parameters, and population growth rate. Microplastics exposure (0.1 mg/l of pristine polymer microspheres 1–5 μm diameter) caused parental mortality (10–100%), and significantly ($p \leq 0.05$) decreased growth, reproduction, and population growth rate leading to the extinction of the microplastics-exposed model population in the F_1 generation. Females descending from those exposed to microplastics in F_0 and exposed to clean medium presented some recovery but up to the F_3 generation they still had significantly ($p \leq 0.05$) reduced growth, reproduction, and population growth rate. Overall, these results indicate that *D. magna* recovery from chronic exposure to microplastics may take several generations, and that the continuous exposure over generations to microplastics may cause population extinction. These findings have implications to aquatic ecosystem functioning and services, and raise concern on the long-term animal and human exposure to microplastics through diverse routes.

1. Introduction

Freshwater ecosystems provide most important services to the human society, such as water for drinking and several domestic, agricultural and industrial uses, species for human consumption, climate

* Corresponding author at: ICBAS – Institute of Biomedical Sciences of the University of Porto, Department of Populations Study, Laboratory of Ecotoxicology (ECOTOX), Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.
E-mail address: lguilher@icbas.up.pt (L. Guilhermino).

https://doi.org/10.1016/j.scitotenv.2018.03.054
0048-9697/

regulation, among several others. Their biodiversity and functioning that are crucial for the sustainability of life on earth are threatened by several pressures, such as alterations due to global changes, over-exploration of resources, and contamination by a high number of chemical substances. Among such contaminants, plastics, microplastics and nanoplastics, have been raising high concern regarding environmental and human health (Wagner et al., 2014; Ferreira et al., 2016; Fonte et al., 2016; Horton et al., 2017; Richardson and Ternes, 2018; Wright and Kelly, 2017). The studies performed in recent years revealed that microplastics are widespread in freshwater ecosystems (Eerkes-Medrano et al., 2015; Anderson et al., 2016; Baldwin et al., 2016; Duis and Coors, 2016; Horton et al., 2017; Lebreton et al., 2017; Leslie et al., 2017; Peng et al., 2018; Richardson and Ternes, 2018). The levels of microplastics in freshwaters are diverse but very high abundances and concentrations have been found in polluted areas, such as 44,435 particles Km^{-2} in the Lake Hovsgol, Mongolia (Free et al., 2014), 141,647.7 particles 1000 m^{-3} in the Danube River, Austria (Lechner et al., 2014), 1,146,418.36 particles m^{-3} in the Los Angeles River, USA (Moore et al., 2011), average abundance of 892,777 particles Km^{-2} in the Rhine River (Mani et al., 2015), and average concentrations of 1.56 $\pm$ 1.64 and 5.51 $\pm$ 9.09 mg/l in lakes and wetlands, respectively, of Texas, USA (Lasee et al., 2017). Freshwater animals of different trophic levels ingest microplastics, including species consumed as food by humans (Phillips and Bonner, 2015; Peters and Bratton, 2016). Exposure of freshwater organisms to microplastics and nanoplastics has been found to cause mortality, neurotoxicity, oxidative stress and damage, decrease of individual and population fitness, and several other adverse effects (Au et al., 2015; Bhattacharya et al., 2010; Lagarde et al., 2016; Libralato et al., 2017; Horton et al., 2017; Ziajahromi et al., 2017; Guilhermino et al., 2018). However, the long-term biological and ecological effects of microplastics and nanoplastics are still poorly understood. Such knowledge is most important to assess environmental and human health risks and to increase safety in the use and management of these particles (Syberg et al., 2015; Ferreira et al., 2016; Fonte et al., 2016; Horton et al., 2017; Guilhermino et al., 2018).

In freshwater ecosystems as in other aquatic ecosystems, the zooplankton community plays a determinant role in ecosystem functioning especially by controlling the phytoplankton community contributing to prevent eutrophication and providing food to upper trophic levels, including several species of human consumption. Thus, investigating the effects of pressures on zooplankton organisms and populations provides valuable knowledge on adverse alterations that may affect the whole ecosystem.

Daphnia magna is one of the freshwater zooplankton species that have been most studied in relation to environmental contaminants and other stressors. This species is able to ingest microplastics of different types and sizes, including long synthetic fibers around 300 μm and even longer ones (Jemec et al., 2016; Frydkjær et al., 2017). In *D. magna*, microplastics and nanoplastics have been found to cause a wide range of adverse effects, such as immobilization, mortality, feeding inhibition, decrease of the reproductive fitness, among several other (Besseling et al., 2014; Jemec et al., 2016; Nasser and Lynch, 2016; Ma et al., 2016; Ogonowski et al., 2016; Rehse et al., 2016; Frydkjær et al., 2017; Kim et al., 2017). Despite the importance of the knowledge provided by the research already done, more knowledge on transgenerational effects and recovery of microplastics exposure is needed to understand the impacts that microplastics may have on *D. magna* populations, zooplankton communities and ecosystem functioning.

In the present study, the transgenerational effects of microplastics and post-exposure recovery were investigated in *D. magna* model populations. This species was selected mainly because has a long record of use in ecotoxicology as representative of freshwater zooplankton species, is a convenient model to use in long-term studies, and the bioassays with this species were proposed for use as pre-screening of toxicity to humans and other mammals (Baird et al., 1989a, 1989b; Rehse et al., 2016; Guilhermino et al., 1999, 2000; Martins et al., 2013; Pacheco et al., 2018).

2. Material and methods

2.1. Chemicals

The chemicals (analytical grade) used to prepare culture and test media for *D. magna* and culture media for *Chlorella vulgaris* (used as food for *D. magna*) were from Sigma Aldrich (Germany) or Merck (Germany). Microplastics were red fluorescent polymer microspheres purchased from Cospheric Innovations in Microtechnology (U.S.A.), lot number: 4-1006-1053, provided as dry powder. According the manufacturer indications particles had 1–5 μm diameter, excitation and emission wavelengths of 575 nm and 607 nm, respectively, 1.3 g/cm^3 density, and 1 mg of the product contains about 1.836E + 8 spheres (estimate made for an average of 2 μm diameter). Further characterization and behaviour of microplastics over 24 h is provided in Pacheco et al. (2018). These particles were selected mainly because of their low size, their fluorescence allowing the easy quantification in test medium and detection inside *D. magna*, and the wide applications of fluorescent microplastic particles, including in medical and biomedical applications and research. Moreover, the same type of particles was previous tested regarding its toxicity to *D. magna* and considered a suitable model of primary microplastics widely used in cosmetics, personal care products, and several other applications (Ogonowski et al., 2016; Pacheco et al., 2018).

2.2. Parental organisms and general conditions of the bioassays

Test organisms were *Daphnia magna* Straus, clone A *sensus* Baird et al. (1989a), feed with *Chlorella vulgaris.* The cultures of both species were maintained in the Laboratory of Ecotoxicology of ICBAS (ECOTOX), University of Porto, for several years as indicated in Guilhermino et al. (1999).

Bioassays were carried out in a Bronson PGC 1400 chamber (The Netherlands) with control of temperature (20 $\pm$ 1 °C) and photoperiod (16 h light: 8 h dark). Each bioassay had an exposure period of 21 days (punctually 22 days to allow the release of the last brood of juveniles). All bioassays were started with females with >6 h and <24 h old, and followed OECD guidelines (OECD, 2012) with some modifications as further described. Test medium was the American Society for Testing and Materials (ASTM) hard water (ASTM, 1980), hereafter indicated as test medium, supplemented with vitamins and 4 ml/l of *Ascophyllum nodosum* extract (Baird et al., 1989b; Bradley et al., 1993; Guilhermino et al., 1999). It was renewed at each 24 h. Animals were exposed individually in glass beakers (100 ml volume) filled with 50 ml of test solution, covered but allowing some air entrance. Ten animals were used per treatment, and they were feed with *Chlorella vulgaris* (3×10^5 cells/ml/daphnid corresponding to 0.322 mg of carbon/daphnid/day) (Guilhermino et al., 1999).

2.3. Experimental design

Bioassays covered 4 sequential generations, hereafter indicated as F_0, F_1, F_2 and F_3. Three model populations with a common origin were investigated: control population (exposed to clean test medium), microplastics-exposed population (animals exposed to test medium containing 0.1 mg/l of microplastics), and recovery population (animals exposed to microplastics in the F_0 generation; following generations exposed to clean test medium). The concentration of microplastics tested (0.1 mg/l) was selected based on a previous 21-day bioassay assessing the chronic effects of the same particles on *D. magna* (Pacheco et al., 2018).

The experimental design of the bioassays is indicated in Fig. 1, where groups of 10 females exposed to different treatments are indicated by