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Motion behavior and metabolic response to microplastic leachates in the benthic foraminifera *Haynesina germanica*

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Abstract :

Plastic is one of the major sources of pollution in modern oceans. When in seawater, toxic plasticizers (the additives incorporated in plastic polymers during manufacturing processes) typically diffuse and accumulate in sediments and in benthic and pelagic organisms' tissues. These plastic leachates affect survival, behavior and metabolism of various marine metazoans, but little effort was placed in studying their effect on protists. In this contribution we monitored the short-term effect of polypropylene (PP) leachates at both environmentally realistic and chronic concentrations on Haynesina germanica locomotion and metabolism. We found that PP leachates has no lethal nor effects on this species activity. Taken together, these results suggest that benthic foraminifera may be more resistant than marine metazoans to plasticizers pollutants.

Highlights

► Short-term exposure to polypropylene leachates does not affect foraminiferal survival. ► Polypropylene leachates do not change *Haynesina germanica* behavior nor respiration. ► Foraminiferal resistance to plastic pollution might provide a competitive advantage.

Keywords : Benthic foraminifera, Plastic leachates, Polypropylene, Survival, Behavior, Respiration

1 Introduction

Plastics are acknowledged as one of the most ubiquitous and conspicuous sources of pollution of the Anthropocene, especially in the marine environment (W. C. Li et al., 2016). Microplastics (MP) can either be small plastic particles (smaller than 5mm) released in the environment or result from the breakage and aging of macroplastics. They are now considered the most numerically abundant form of solid waste on the planet (Eriksen et al., 2014) and a potential threat to marine ecosystems globally (Galloway et al., 2017). Hence, they are widely observed from coastal waters to the deep-ocean floor and from tropical to polar regions (Barnes, 2005; Chiba et al., 2018).

Microplastics are also responsible for a range of sub-lethal effects related to their pernicious role as a vector of chemical pollutants. These pollutants leaching from MP to the marine environments originate from the additives compounds (e.g. plasticizers, flame retardant, UV stabilizers, antioxidant, and antistatic molecule) incorporated in plastics during the manufacturing process to modify the plastic polymers physical properties and durability, but also from the chemical compounds already present in the water (i.e. coming from another source of pollution) which are adsorbed at the MP's surface when aging in the environments. Plastic additives such as phthalates, bisphenol A, nonyphenols and brominated flame retardants can reach high concentrations in coastal waters (Hermabessiere et al., 2017; Sánchez-Avila et al., 2012) and accumulate in marine organisms tissues (Vered et al., 2019). This work specifically focuses on the toxicity of virgin MP leachates since they have recently been identified as one of the most critical threat related to the presence of plastics in the ocean (Hahladakis et al., 2018; Paluselli et al., 2019). The toxic effects of virgin microplastic leachates have been reported in various marine faunal taxa, such as barnacles (H.-X. Li et al., 2016), crustacean larvae (Lithner et al., 2009), gastropods (Seuront, 2018), bivalves (Ke et al., 2019) and sea urchins (Oliviero et al., 2019). Desorption of these chemicals in the surrounding environment causes a range of harmful effects on embryo development, reproduction, behavior or induce genetic aberrations (see Oehlmann et al. (2009) for a review).

To date and to the best of our knowledge, there is still a critical lack of information available on effect of microplastic on protists, despite a recent urge to fill this knowledge gap (Rillig and Bonkowski, 2018). However, MP ingestion is likely to be common in protists (Setälä et al., 2014), including foraminifera (Ciacci et al., 2019), and subsequently negatively impact their metabolic activity (Ciacci et al., 2019; Su et al., 2020). Benthic foraminifera were targeted in this work due to their importance in the structure and function of benthic ecosystems (Geslin et al., 2011; Gooday et al., 1992), their ability to respond to various types of pollutant both under laboratory conditions (Denoyelle et al., 2012; Ernst et al., 2006; Nigam et al., 2009) and *in situ* (see Alve, 1995 for a review). Like any benthic organisms, they are directly exposed to the range of pollutants, including microplastics (Schwarz et al., 2019) which cannot be degraded by bacteria (Nauendorf et al., 2016) and therefore accumulate in coastal sediments (Galgani et al., 1996). In this context, the present study assessed the potential short-term effects of the leachates from virgin polypropylene pellets considered at both environmentally realistic and chronic concentrations on the stress level of the benthic foraminifera *Haynesina germanica*. Specifically, movement behavior (Seuront, 2018) and respiration rate (Su et al., 2020) were considered as proxies of the stress level of *H. germanica* following an exposure to polypropylene leachates. This foraminiferal species and this plastic polymer were specifically chosen for their high abundances along the French coast of the eastern English Channel (Armynot du Châtelet et al., 2018; Francescangeli et al., 2017; Hermabessiere et al., 2019).

2 Material and methods

2.1 *Haynesina germanica* collection

Surface sediment (0-1cm) from Boulogne-sur-Mer harbor mudflat (eastern English Channel, 50°43'06.4"N 1°34'22.0"E) was sampled in June 2019 and stored in 100 ml polypropylene containers. Sediment was kept at ambient temperature during transportation and placed within one hour in English Channel seawater aquarium (12°C and 35 PSU) under a natural day-light cycle conditions until the experiment took place. Sediment was sieved over a 125 µm stainless-steel mesh and colored-cytoplasm *Haynesina germanica* were subsequently sorted. Only the active specimens (i.e. leaving a displacement track on a thin layer of sediment) were considered as living and selected for the experiment. Living individuals were transferred in artificial seawater (ASW) prepared with 35 grams of sea salt (RedSea Fish Farm, Israel) per liter of Milli-Q water (Merck Millipore, Germany) and gently cleaned with a brush to remove any surrounding particles.

2.2 Experimental conditions

Both behavioral experiments and metabolic measurements were conducted exposing *H. germanica* to artificial seawater as control and to microplastic leachates seawater. Microplastic leachates seawater was prepared from commercially available virgin polypropylene pellets (typically 3.3 to 4.7 mm in diameter; Pemmiproducts, Germany) mixed with artificial seawater at a concentration of 20 ml and 200 ml of pellets per liter (hereafter respectively referred to as PP20 and PP200) and aerated for 24 h before the beginning of the experiments following the protocol developed in Seuront (2018) to monitor the effect of plastic leachates on a marine gastropod. Although not quantified in this experiment, polypropylene leachates typically contain bisphenol A, octylphenol and nonylphenol (Hermabessiere et al., 2017).

2.3 Behavioral experiment

For each experimental condition (i.e. control seawater and the two leachate treatments PP20 and PP200), 15 living *Haynesina germanica* (maximum diameter range: 300-440 µm) were spread randomly on the bottom of 15-cm wide glass-Petri dishes filled with ASW, PP20 or PP200 (Figure 1). Petri dishes were placed in a light and temperature-controlled incubator (MIR-154, Panasonic, Japan) set at 12°C. The movements of *H. germanica* were recorded every 10 minutes using a digital camera

(V1 with a 10-30 mm lens, Nikon, Japan) under homogenous dim light conditions (photosynthetically active radiation <100 µmol photon m^{-2} s^{-1}; SA-190 quantum sensor, LI-COR, USA) provided by a horizontal array of LEDs (YN-160 III, Yongnuo, China). Each experiment lasted 10 hours.

Images were compiled in the open-source image analysis software Fiji (Schindelin et al., 2012) and (*x,y*) coordinates were measured for each individual *H. germanica* using the *Manual Tracking* plugin (Figure 1). The distance travelled (D_t) by each individual between two images was calculated as: $D_t = \sqrt{[(x_t - x_{t+1})^2 + (y_t - y_{t+10})^2]}$ where (x_t, y_t) and (x_{t+10}, y_{t+10}) are the coordinates between two successive images taken at 10-minute intervals. The total distance travelled in 10 hours was calculated from the sum of all D_t and subsequently converted to locomotion speed (mm h^{-1}). These behavioral parameters were measured using *trajr* package (McLean and Skowron Volponi, 2018) in R v.3.5.3 (R Core Team, 2019). Trajectories complexity was assessed using fractal analysis. The fractal dimensions of foraminifera trajectories were estimated following the box dimension method (Seuront, 2015, 2010).

2.4 Respiration measurements

Five *H. germanica* specimens were randomly selected from the individuals used in behavioral experiments and transferred from the Petri dish to a 1-mm wide glass microtube containing the three tested seawater (ASW for control and PP20 and PP200 to test the effect of polypropylene leachates). Steady-state oxygen consumption gradient (dC/dz, in pmol cm^{-4}) in the millimeter above the organisms were measured using a 50-µm Clark-type oxygen microelectrode (Unisense, Denmark). Oxygen fluxes (J, pmol cm^{-1} s^{-1}) in the microtube were calculated using Fick's first law of free diffusion as $J = D \times dC/dz$ (Li and Gregory, 1974) with D being the free diffusion coefficient for oxygen ($D = 1.6\ 10^{-5}$ cm^2 s^{-1} at 12°C and 35PSU). Individual respiration rate (R, pmol ind^{-1} day^{-1}) was then calculated as $R = J \times S/n$ (considering the microtube inner section $S = 7.9\ 10^{-3}$ cm^2 and the number of individuals $n = 5$). Note that our measurements were conducted on groups of 5 individuals both to take into account the low individual respiration rate of benthic foraminifera and to overpass the sensor detection limit (Geslin et al., 2011). Respiration rate measurements were replicated 6 times in control seawater and triplicated in both P20 and PP200 leachate treatments. Since respiration is influenced by individual size, specimens were measured to normalize the respiration rates by the foraminiferal biovolume (8.10^6 μm^3 in average; estimated following Geslin et al., 2011). All respiration measurements were carried out in the dark in a 12°C temperature-controlled water bath (Huber CC-K12, Germany).

2.5 Data analysis

Due to our small size samples, the effect of the 3 experimental conditions on movement speed, fractal dimension and foraminiferal respiration rate was tested using Kruskal-Wallis test (Hollander and Wolfe, 1999) in R v.3.5.3 (R Core Team, 2019).

3 Results

Image analysis show that 100% of the individuals tested were moving throughout the experiments and were still alive after being exposed to PP20 and PP200 for 10 hours. *Haynesina germanica* moved over distances ranging from 7 to 32 mm, at locomotion speed ranging from 0.7 to 3.2 mm h^{-1}, 1.6 to 2.8 mm h^{-1} and 1.1 to 3.1 mm h^{-1} for ASW, PP20 and PP200 respectively (Figure 1A). All the trajectories considered in this work were significantly described in terms of fractal dimensions that ranged between 1.02 and 1.13 with average values of 1.07, 1.06 and 1.06 in ASW, PP20 and PP200 respectively (Figure 1B). Finally, respiration rate ranged from 41 to 114 10^{-6} pmol μm^{-3} day^{-1} in the ASW control, from 66 to 164 10^{-6} pmol μm^{-3} day^{-1} in PP20 and from 84 to 98 10^{-6} pmol μm^{-3} day^{-1} in PP200 (Figure 1C). Neither locomotion speed, fractal dimensions nor respiration rates exhibited any significant differences between the three experimental conditions (Kruskall-Wallis-test: $p>0.05$; Table 1).

4 Discussion

The additives leaching from polypropylene (i.e. essentially antioxidant additives such as bisphenol A, octylphenol and nonylphenol; Hermabessiere et al., 2017) have lethal effects on mollusks (Oehlmann et al., 2000), barnacle larvae (H.-X. Li et al., 2016), amphibians (Hogan et al., 2006), annelids and crustaceans (Staples et al., 2016). In contrast, the present work showed a lack of any lethal effect on *Haynesina germanica* of PP leachates.

Similarly, no sublethal effect were perceptible through *H. germanica* locomotion and metabolism. Specifically, locomotion speed was nearly 2-fold lower than those reported previously on the same species (here ~2 mm h^{-1} *vs.* ~4 mm h^{-1} in Seuront and Bouchet, 2015) probably due to the lower experimental temperature (12°C here *vs.* 22°C in Seuront and Bouchet, 2015) since decreasing temperature is known to reduce foraminiferal activity (Bradshaw, 1961). Our results nevertheless clearly indicated that PP leachates did not affect foraminiferal behavior (Figure 1A, B). This is consistent with the observed lack of behavioral impairment in the intertidal gastropod *Littorina littorea*; as PP20 leachates impaired their chemosensory ability without impacting their neuromuscular abilities (Seuront, 2018). In turn, our results contrast with previous evidence that PP-plasticizers reduce fish larvae velocity in the first days after hatching (Inagaki et al., 2016; Wang et al., 2013) and negatively impact adult-fish locomotion and reproductive behavior after at least 2 months of exposure (Gray et al., 1999; Xia et al., 2010). Note that the apparent discrepancy observed between the aforementioned studies and our experiment might be due to differences in exposure duration as reported in Table 2.

Plasticizers have previously been reported to lead to an immediate increase followed by a decrease in respiration rates with rising phenols concentration in mollusks (Levine and Cheney, 2000). They can also induce energetical impairments in crustaceans anaerobic metabolism in less than 2 days (Nagato et al., 2016). In contrast, we did not find any significant effects of PP leachates on foraminiferal respiration, even under very high leachates concentrations, i.e. PP200 (Figure 1C). To the best of our knowledge, the only other study that investigated the effect of PP leachates on a unicellular organism found a decrease in dinoflagellate photosynthesis (M'Rabet et al., 2018) after 1 day of exposure, in accordance with the reduced growth and oxygen production observed in the marine cyanobacteria *Prochlorococcus* following a 24h-long exposure to leachates of common plastic items (i.e. HDPE shopping bags and PVC matting; Tetu et al., 2019). Note that, conversely to M'Rabet et al. (2018) who specifically worked with bisphenol A (Table 2), we did not have any control on the composition of the PP leachates. This is a clear limitation of our study that will need to be improved in future works.

Overall, both the behavioral and metabolic activity data gathered in this preliminary study indicate that the benthic foraminifera *Haynesina germanica* do not respond to MP unlike other unicellular and metazoan organisms. Though this is highly speculative, this observation may suggest that their resistance to leachates from virgin PP might induce a competitive advantage for benthic foraminifera. Such a competitive advantage for foraminifera has previously been observed in relation to some anthropo-natural phenomena such as organic-matter enrichment and anoxia (Langlet et al., 2013; Stachowitsch, 2014). Note, however, that the observed lack of effect of MP on foraminiferal activity may also be due to the relatively short-term exposure used in our experiments. More fundamentally, the diversity of methods reported in the literature related to the type of polymer considered, the use of unidentified leachates or a specific plasticizer, the pollutant concentrations, the duration of exposure as well as the biology of the organisms considered (Table 2) dramatically prevent to reach a general consensus when comparing the effect of MP on foraminifera with other organisms in our study. In this context, future experiments aiming to assess the effects of MP leachates on benthic foraminifera should benefit from (i) being more specific about the acute or chronic nature of their exposure, and (ii) identifying and quantifying the plasticizers used or present in the leachates. Finally, further work is also needed to assess the potential effects of leachates from (i) weathered PP in particular as they have shown to have significantly stronger effects that virgin plastics (Bejgarn et al., 2015; Gandara e Silva et al., 2016; Kedzierski et al., 2018; Nobre et al., 2015; Seuront, 2018), (ii) different plastic polymers (H.-X. Li et al., 2016; Lithner et al., 2012, 2009; Tetu et al., 2019) and (iii) ingested plastic particles (Ciacci et al., 2019).

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References cited

Alve, E., 1995. Benthic foraminiferal responses to estuarine pollution: a review. J. Foraminifer. Res. 25, 190–203.

Armynot du Châtelet, E., Francescangeli, F., Bouchet, V.M.P., Frontalini, F., 2018. Benthic foraminifera in transitional environments in the English Channel and the southern North Sea: A proxy for regional-scale environmental and paleo-environmental characterisations. Mar. Environ. Res. 137, 37–48. https://doi.org/10.1016/j.marenvres.2018.02.021

Barnes, D.K.A., 2005. Remote Islands Reveal Rapid Rise of Southern Hemisphere Sea Debris. Sci. World J. 5, 915–921. https://doi.org/10.1100/tsw.2005.120

Bejgarn, S., MacLeod, M., Bogdal, C., Breitholtz, M., 2015. Toxicity of leachate from weathering plastics: An exploratory screening study with *Nitocra spinipes*. Chemosphere 132, 114–119. https://doi.org/10.1016/j.chemosphere.2015.03.010

Bradshaw, J.S., 1961. Laboratory experiment on the ecology of foraminifera. Contrib. Cushman Found. Foraminifer. Res. 12, 87–106.

Chiba, S., Saito, H., Fletcher, R., Yogi, T., Kayo, M., Miyagi, S., Ogido, M., Fujikura, K., 2018. Human footprint in the abyss: 30 year records of deep-sea plastic debris. Mar. Policy 96, 204–212. https://doi.org/10.1016/j.marpol.2018.03.022

Ciacci, C., Grimmelpont, M.V., Corsi, I., Bergami, E., Curzi, D., Burini, D., Bouchet, V.M.P., Ambrogini, P., Gobbi, P., Ujiié, Y., Ishitani, Y., Coccioni, R., Bernhard, J.M., Frontalini, F., 2019. Nanoparticle-Biological Interactions in a Marine Benthic Foraminifer. Sci. Rep. 9, 19441. https://doi.org/10.1038/s41598-019-56037-2

Denoyelle, M., Geslin, E., Jorissen, F.J., Cazes, L., Galgani, F., 2012. Innovative use of foraminifera in ecotoxicology: A marine chronic bioassay for testing potential toxicity of drilling muds. Ecol. Indic., Marine Benthic Indicators 12, 17–25. https://doi.org/10.1016/j.ecolind.2011.05.011

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLOS ONE 9, e111913. https://doi.org/10.1371/journal.pone.0111913

Ernst, S.R., Morvan, J., Geslin, E., Le Bihan, A., Jorissen, F.J., 2006. Benthic foraminiferal response to experimentally induced Erika oil pollution. Mar. Micropaleontol. 61, 76–93. https://doi.org/10.1016/j.marmicro.2006.05.005

Francescangeli, F., Bouchet, V.M.P., Trentesaux, A., Armynot du Chatelet, E., 2017. Does elevation matter? Living foraminiferal distribution in a hyper tidal salt marsh (Canche Estuary, Northern France). Estuar. Coast. Shelf Sci. 194, 192–204. https://doi.org/10.1016/j.ecss.2017.06.023

Galgani, F., Souplet, A., Cadiou, Y., 1996. Accumulation of debris on the deep sea floor off the French Mediterranean coast. Mar. Ecol. Prog. Ser. 142, 225–234. https://doi.org/10.3354/meps142225

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1, 0116. https://doi.org/10.1038/s41559-017-0116

Gandara e Silva, P.P., Nobre, C.R., Resaffe, P., Pereira, C.D.S., Gusmão, F., 2016. Leachate from microplastics impairs larval development in brown mussels. Water Res. 106, 364–370. https://doi.org/10.1016/j.watres.2016.10.016

Geslin, E., Risgaard-Petersen, N., Lombard, F., Metzger, E., Langlet, D., Jorissen, F., 2011. Oxygen respiration rates of benthic foraminifera as measured with oxygen microsensors. J. Exp. Mar. Biol. Ecol. 396, 108–114. https://doi.org/10.1016/j.jembe.2010.10.011

Gooday, A.J., Levin, L.A., Linke, P., Heeger, T., 1992. The Role of Benthic Foraminifera in Deep-Sea Food Webs and Carbon Cycling, in: Rowe, G.T., Pariente, V. (Eds.), Deep-Sea Food Chains and the Global Carbon Cycle, NATO ASI Series. Springer Netherlands, pp. 63–91.

Gray, M.A., Teather, K.L., Metcalfe, C.D., 1999. Reproductive success and behavior of Japanese medaka (*Oryzias latipes*) exposed to 4-tert-octylphenol. Environ. Toxicol. Chem. 18, 2587–2594. https://doi.org/10.1002/etc.5620181128

Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard. Mater. 344, 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017. Occurrence and effects of plastic additives on marine environments and organisms: A review. Chemosphere 182, 781–793. https://doi.org/10.1016/j.chemosphere.2017.05.096

Hermabessiere, L., Paul-Pont, I., Cassone, A.-L., Himber, C., Receveur, J., Jezequel, R., El Rakwe, M., Rinnert, E., Rivière, G., Lambert, C., Huvet, A., Dehaut, A., Duflos, G., Soudant, P., 2019. Microplastic contamination and pollutant levels in mussels and cockles collected along the channel coasts. Environ. Pollut. 250, 807–819. https://doi.org/10.1016/j.envpol.2019.04.051

Hogan, N.S., Lean, D.R.S., Trudeau, V.L., 2006. Exposures to Estradiol, Ethinylestradiol and Octylphenol Affect Survival and Growth of *Rana pipiens* and *Rana sylvatica* Tadpoles. J. Toxicol. Environ. Health A 69, 1555–1569. https://doi.org/10.1080/15287390500470759

Hollander, M., Wolfe, D.A., 1999. Nonparametric Statistical Methods. Wiley.

Inagaki, T., Smith, N., Lee, E.K., Ramakrishnan, S., 2016. Low dose exposure to Bisphenol A alters development of gonadotropin-releasing hormone 3 neurons and larval locomotor behavior in Japanese Medaka. NeuroToxicology 52, 188–197. https://doi.org/10.1016/j.neuro.2015.12.003

Ke, A.-Y., Chen, J., Zhu, J., Wang, Y.-H., Hu, Y., Fan, Z.-L., Chen, M., Peng, P., Jiang, S.-W., Xu, X.-R., Li, H.-X., 2019. Impacts of leachates from single-use polyethylene plastic bags on the early development of clam *Meretrix meretrix* (Bivalvia: Veneridae). Mar. Pollut. Bull. 142, 54–57. https://doi.org/10.1016/j.marpolbul.2019.03.029

Kedzierski, M., D'Almeida, M., Magueresse, A., Le Grand, A., Duval, H., César, G., Sire, O., Bruzaud, S., Le Tilly, V., 2018. Threat of plastic ageing in marine environment. Adsorption/desorption of micropollutants. Mar. Pollut. Bull. 127, 684–694. https://doi.org/10.1016/j.marpolbul.2017.12.059

Langlet, D., Geslin, E., Baal, C., Metzger, E., Lejzerowicz, F., Riedel, B., Zuschin, M., Pawlowski, J., Stachowitsch, M., Jorissen, F.J., 2013. Foraminiferal survival after long-term in situ experimentally induced anoxia. Biogeosciences 10, 7463–7480. https://doi.org/10.5194/bg-10-7463-2013

Levine, C., Cheney, M.A., 2000. Metabolic responses to acute toxicity of alkylphenols and alkylphenol polyethoxylates in *Elliptio complanata* measured by calorespirometry. Environ. Toxicol. Chem. 19, 1906–1910. https://doi.org/10.1002/etc.5620190727

Li, H.-X., Getzinger, G.J., Ferguson, P.L., Orihuela, B., Zhu, M., Rittschof, D., 2016. Effects of Toxic Leachate from Commercial Plastics on Larval Survival and Settlement of the Barnacle *Amphibalanus amphitrite*. Environ. Sci. Technol. 50, 924–931. https://doi.org/10.1021/acs.est.5b02781

Li, W.C., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects. Sci. Total Environ. 566–567, 333–349. https://doi.org/10.1016/j.scitotenv.2016.05.084

Li, Y.-H., Gregory, S., 1974. Diffusion of ions in sea water and in deep-sea sediments. Geochim. Cosmochim. Acta 38, 703–714. https://doi.org/10.1016/0016-7037(74)90145-8

Lithner, D., Damberg, J., Dave, G., Larsson, Å., 2009. Leachates from plastic consumer products – Screening for toxicity with *Daphnia magna*. Chemosphere 74, 1195–1200. https://doi.org/10.1016/j.chemosphere.2008.11.022

Lithner, D., Nordensvan, I., Dave, G., 2012. Comparative acute toxicity of leachates from plastic products made of polypropylene, polyethylene, PVC, acrylonitrile–butadiene–styrene, and epoxy to *Daphnia magna*. Environ. Sci. Pollut. Res. 19, 1763–1772. https://doi.org/10.1007/s11356-011-0663-5

McLean, D.J., Skowron Volponi, M.A., 2018. trajr : An R package for characterisation of animal trajectories. Ethology 124, 440–448. https://doi.org/10.1111/eth.12739

M'Rabet, C., Pringault, O., Zmerli-Triki, H., Ben Gharbia, H., Couet, D., Kéfi-Daly Yahia, O., 2018. Impact of two plastic-derived chemicals, the Bisphenol A and the di-2-ethylhexyl phthalate, exposure on the marine toxic dinoflagellate Alexandrium pacificum. Mar. Pollut. Bull. 126, 241–249. https://doi.org/10.1016/j.marpolbul.2017.10.090

Nagato, E.G., Simpson, A.J., Simpson, M.J., 2016. Metabolomics reveals energetic impairments in *Daphnia magna* exposed to diazinon, malathion and bisphenol-A. Aquat. Toxicol. 170, 175–186. https://doi.org/10.1016/j.aquatox.2015.11.023

Nauendorf, A., Krause, S., Bigalke, N.K., Gorb, E.V., Gorb, S.N., Haeckel, M., Wahl, M., Treude, T., 2016. Microbial colonization and degradation of polyethylene and biodegradable plastic bags in temperate fine-grained organic-rich marine sediments. Mar. Pollut. Bull. 11.

Nigam, R., Linshy, V.N., Kurtarkar, S.R., Saraswat, R., 2009. Effects of sudden stress due to heavy metal mercury on benthic foraminifer *Rosalina leei*: Laboratory culture experiment. Mar. Pollut. Bull., Foraminifera and Marine Pollution 59, 362–368. https://doi.org/10.1016/j.marpolbul.2009.08.014

Nobre, C.R., Santana, M.F.M., Maluf, A., Cortez, F.S., Cesar, A., Pereira, C.D.S., Turra, A., 2015. Assessment of microplastic toxicity to embryonic development of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). Mar. Pollut. Bull. 92, 99–104. https://doi.org/10.1016/j.marpolbul.2014.12.050

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look, K.J.W., Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2047–2062. https://doi.org/10.1098/rstb.2008.0242

Oehlmann, J., Schulte-Oehlmann, U., Tillmann, M., Markert, B., 2000. Effects of Endocrine Disruptors on Prosobranch Snails (Mollusca: Gastropoda) in the Laboratory. Part I: Bisphenol A and Octylphenol as Xeno-Estrogens. Ecotoxicology 9, 383–397. https://doi.org/10.1023/A:1008972518019

Oliviero, M., Tato, T., Schiavo, S., Fernández, V., Manzo, S., Beiras, R., 2019. Leachates of micronized plastic toys provoke embryotoxic effects upon sea urchin *Paracentrotus lividus*. Environ. Pollut. 247, 706–715. https://doi.org/10.1016/j.envpol.2019.01.098

Paluselli, A., Fauvelle, V., Galgani, F., Sempéré, R., 2019. Phthalate Release from Plastic Fragments and Degradation in Seawater. Environ. Sci. Technol. 53, 166–175. https://doi.org/10.1021/acs.est.8b05083

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rillig, M.C., Bonkowski, M., 2018. Microplastic and soil protists: A call for research. Environ. Pollut. 241, 1128–1131. https://doi.org/10.1016/j.envpol.2018.04.147

Sánchez-Avila, J., Tauler, R., Lacorte, S., 2012. Organic micropollutants in coastal waters from NW Mediterranean Sea: Sources distribution and potential risk. Environ. Int. 46, 50–62. https://doi.org/10.1016/j.envint.2012.04.013

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019

Schwarz, A.E., Ligthart, T.N., Boukris, E., van Harmelen, T., 2019. Sources, transport, and accumulation of different types of plastic litter in aquatic environments: A review study. Mar. Pollut. Bull. 143, 92–100. https://doi.org/10.1016/j.marpolbul.2019.04.029

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013

Seuront, L., 2018. Microplastic leachates impair behavioural vigilance and predator avoidance in a temperate intertidal gastropod. Biol. Lett. 14, 20180453. https://doi.org/10.1098/rsbl.2018.0453

Seuront, L., 2015. On uses, misuses and potential abuses of fractal analysis in zooplankton behavioral studies: A review, a critique and a few recommendations. Phys. A 25.

Seuront, L., 2010. Fractals and Multifractals in Ecology and Aquatic Science, 1st ed. CRC Press, Boca Raton.
Seuront, L., Bouchet, V.M.P., 2015. The devil lies in details: new insights into the behavioural ecology of intertidal foraminifera. J. Foraminifer. Res. 45, 390–401. https://doi.org/10.2113/gsjfr.45.4.390
Stachowitsch, M., 2014. Coastal hypoxia and anoxia: a multi-tiered holistic approach. Biogeosciences 11, 2281–2285.
Staples, C., Mihaich, E., Ortego, L., Caspers, N., Klečka, G., Woelz, J., Hentges, S., 2016. Characterizing the effects of bisphenol A on sediment-dwelling benthic organisms. Environ. Toxicol. Chem. 35, 652–659. https://doi.org/10.1002/etc.3217
Su, Y., Zhang, K., Zhou, Z., Wang, J., Yang, X., Tang, J., Li, H., Lin, S., 2020. Microplastic exposure represses the growth of endosymbiotic dinoflagellate *Cladocopium goreaui* in culture through affecting its apoptosis and metabolism. Chemosphere 244, 125485. https://doi.org/10.1016/j.chemosphere.2019.125485
Tetu, S.G., Sarker, I., Schrameyer, V., Pickford, R., Elbourne, L.D.H., Moore, L.R., Paulsen, I.T., 2019. Plastic leachates impair growth and oxygen production in *Prochlorococcus*, the ocean's most abundant photosynthetic bacteria. Commun. Biol. 2, 184. https://doi.org/10.1038/s42003-019-0410-x
Vered, G., Kaplan, A., Avisar, D., Shenkar, N., 2019. Using solitary ascidians to assess microplastic and phthalate plasticizers pollution among marine biota: A case study of the Eastern Mediterranean and Red Sea. Mar. Pollut. Bull. 138, 618–625. https://doi.org/10.1016/j.marpolbul.2018.12.013
Wang, X., Dong, Q., Chen, Y., Jiang, H., Xiao, Q., Wang, Y., Li, W., Bai, C., Huang, C., Yang, D., 2013. Bisphenol A affects axonal growth, musculature and motor behavior in developing zebrafish. Aquat. Toxicol. 142–143, 104–113. https://doi.org/10.1016/j.aquatox.2013.07.011
Xia, J., Niu, C., Pei, X., 2010. Effects of chronic exposure to nonylphenol on locomotor activity and social behavior in zebrafish (*Danio rerio*). J. Environ. Sci. 22, 1435–1440. https://doi.org/10.1016/S1001-0742(09)60272-2

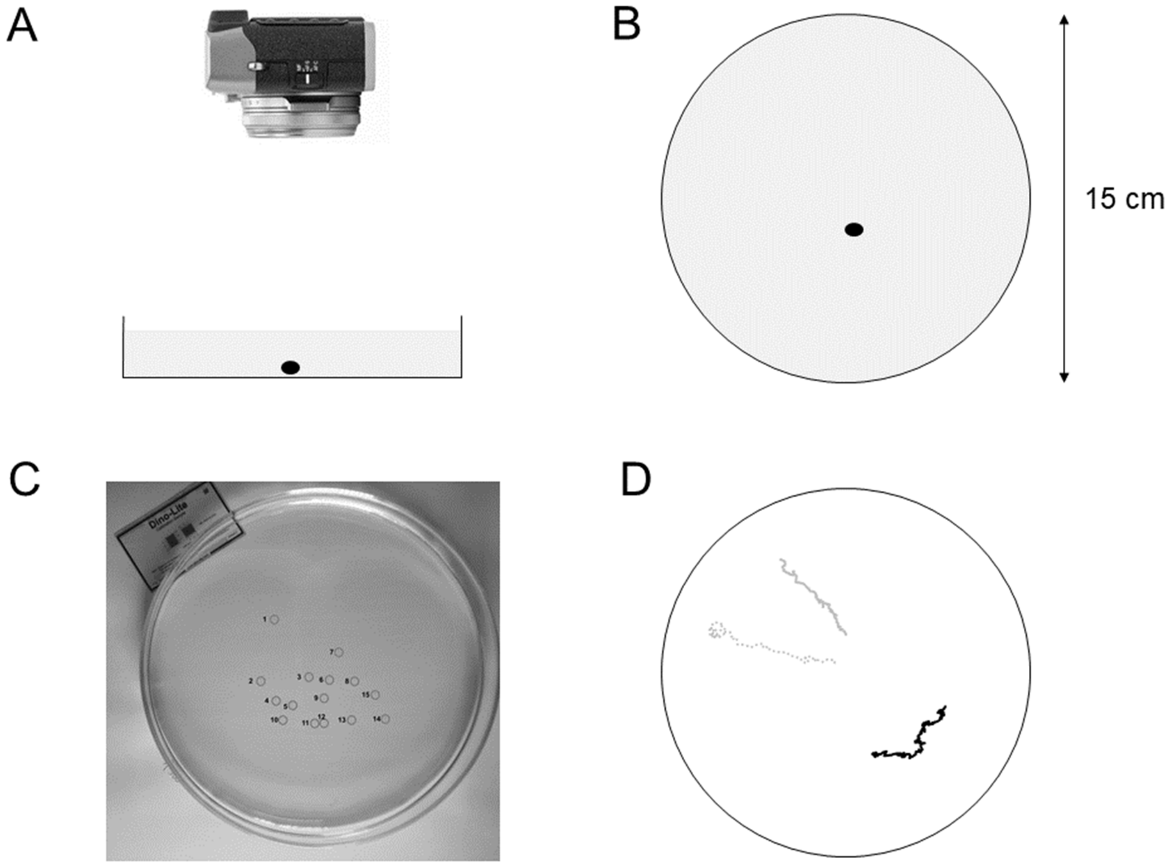
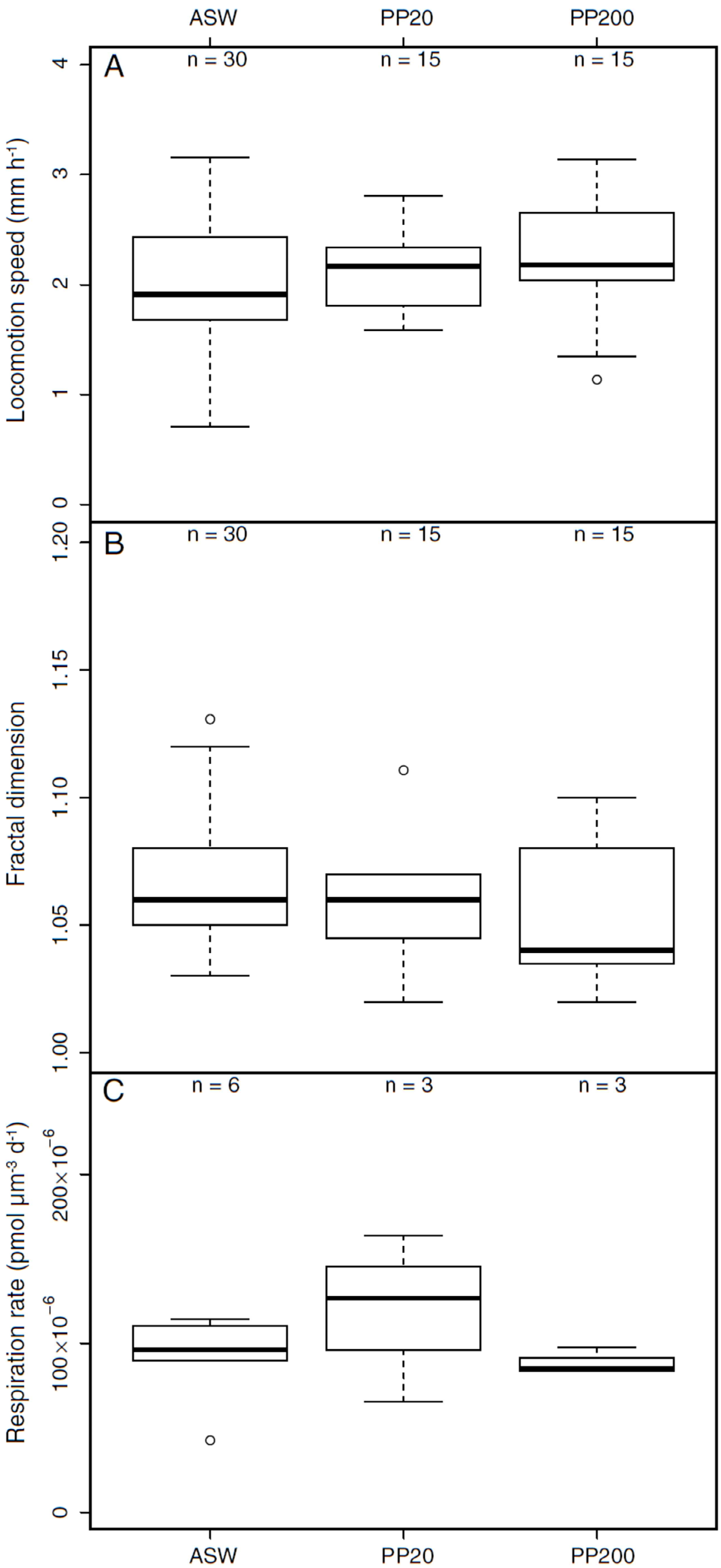


Figure 1 caption: A and B: schematic representation of the experimental setup with lateral view (A) and top view (B) of the position of the foraminifera (black ovoid shape) placed on the petri-dish. C: photograph of the initial position of the 15 individuals used in ASW control conditions. D: example of 3 extracted trajectories for ASW (full black line), PP20 (full grey line) and PP200 (dotted grey line).


ASW
PP20
PP200
A
n = 30
n = 15
n = 15
Locomotion speed (mm h-1)
0
1
2
3
4
B
n = 30
n = 15
n = 15
Fractal dimension
1.00
1.05
1.10
1.15
1.20
C
n = 6
n = 3
n = 3
Respiration rate (pmol µm-3 d-1)
0
100×10−6
200×10−6
ASW
PP20
PP200

410

Figure 2: Locomotion speed (A), fractal dimension (B) and respiration rate (C) of *Haynesina germanica* under the three experimental conditions (ASW: artificial seawater, i.e. control conditions; PP20 and PP200: seawater prepared with 20 and 200 ml l^{-1} polypropylene pellets, respectively). The box represents the first, second and third quartiles and the whiskers extend to 1.5 times the interquartile range. Values outside of this range are represented by open circles.

Table 1 caption: results of the Kruskal-Wallis statistical analyses testing the effect of the experimental conditions (ASW as a control, PP20 and PP200) on the three measured response variables.

Response variable	Kruskal-Wallis X^2	degrees of freedom	p-value
Locomotion speed	1.6	2	0.44
Fractal dimension	3.9	2	0.14
Respiration rate	1.3	2	0.52

Table 2 caption: organisms, response observed, type of pollutant, concentration, equivalent concentration in the present study and exposure duration tested in the literature cited in this article's discussion.

Reference	Organisms	Response observed	Pollutant type	Pollutant concentration	This study's equivalent	Exposure duration
Oehlmann et al. 2000	Mollusks	Mortality	Bisphenol A	1µg/L		5 months
		Mortality	Octylphenol	1µg/L		5 months
Li et al. 2016	Barnacle larvae	10% mortality	PP leachate	0.1 m^2/L	PP200 ~ 0.17m^2/L	1 day
Hogan et al. 2006	Amphibians	50% mortality	Octylphenol	1.4 µmol/L		2 weeks
Staples et al. 2016	Crustaceans	Mortality	Bisphenol A	78 mg/kg sedim dry weight		1 month
	Annelids	Mortality	Bisphenol A	60 mg/kg sedim dry weight		1 month
Seuront 2018	Gastropods	Behavior	PP leachate	20mL/L	PP20 = 20mL/L	3 hours
Inagaki et al. 2016	Fish larvae	Locomotion	Bisphenol A	200ng/mL		20 days
Wang et al. 2013	Fish larvae	Locomotion	Bisphenol A	15µmol/L		2 days
Gray et al. 1999	Adult fish	Reproductive behavior	Octylphenol	25µg/L		3 months
Xia et al. 2010	Adult fish	Locomotion	Nonylphenol	100µg/L		2 months
Levine and Cheney 2000	Mollusks	Respiration	Nonylphenol	10µmol/L		1 hour
Nagato et al. 2016	Crustaceans	Anaerobic metabolism	Bisphenol A	0.1mg/L		2 days
M'Rabet et al. 2018	Dinoflagellate	Respiration and photosynthesis	Bisphenol A	2µg/L		1 day
Tetu et al. 2019	Cyanobacteria	Photosynthesis	PVC leachate	1g/L	PP20 ~ 10g/L	3 hours