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Effects of polypropylene nanofibers on soft corals

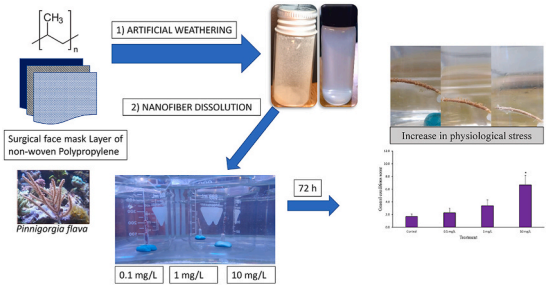
Valerio Isa [a,b,c], Alessandro Becchi [a], Imogen Ellen Napper [d], Paolo Giuseppe Ubaldi [a], Francesco Saliu [a,*], Silvia Lavorano [c], Paolo Galli [a,b,e]

[a] Earth and Environmental Science Department, University of Milano Bicocca, MI, 20126, Italy
[b] MaRHE Center (Marine Research and High Education Center), Magoodhoo Island, Faafu Atoll, Maldives
[c] Costa Edutainment SpA - Acquario di Genova, GE, 16128, Italy
[d] International Marine Litter Research Unit, School of Biological and Marine Sciences, University of Plymouth, Drake's Circus, Plymouth, PL4 8AA, UK
[e] University of Dubai, Dubai, P.O. Box 14143, United Arab Emirates

HIGHLIGHTS

- UV aging of nonwoven polypropylene produced secondary nanofibers.
- Short term exposure of nanofibers to Pinnigorgia flava was assessed.
- No mortality was detected after exposure, yet stress (e.g. mucus production).
- Nanofibers caused coral tissue necrosis in individuals exposed to the 10 mg/L.
- The no observed effect concentration (NOEC) was 0.1 mg/L.

GRAPHICAL ABSTRACT



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ABSTRACT

Current information regarding the effects of both micro- and nano-plastic debris on coral reefs is limited; especially the toxicity onto corals from nano-plastics originating from secondary sources such as fibers from synthetic fabrics. Within this study, we exposed the alcyonacean coral *Pinnigorgia flava* to different concentrations of polypropylene secondary nanofibers (0.001, 0.1, 1.0 and 10 mg/L) and then assayed mortality, mucus production, polyps retraction, coral tissue bleaching, and swelling. The assay materials were obtained by artificially weathering nonwoven fabrics retrieved from commercially available personal protective equipment. Specifically, polypropylene (PP) nanofibers displaying a hydrodynamic size of 114.7 ± 8.1 nm and a polydispersity index (PDI) of 0.431 were obtained after 180 h exposition in a UV light aging chamber (340 nm at 0.76 $W \cdot m^{-2} \cdot nm^{-1}$). After 72 h of PP exposure no mortality was observed but there were evident stress responses from the corals tested. Specifically, the application of nanofibers at different concentrations caused significant differences in mucus production, polyps retraction and coral tissue swelling (ANOVA, $p < 0.001$, $p = 0.015$ and $p = 0.015$, respectively). NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect concentration) at 72 h resulted 0.1 mg/L and 1 mg/L, respectively. Overall, the study indicates that PP secondary nanofibers can cause adverse effects on corals and could potentially act as a stress factor in coral reefs. The generality of the method of producing and assaying the toxicity of secondary nanofibers from synthetic textiles is also discussed.

* Corresponding author.
E-mail address: francesco.saliu@unimib.it (F. Saliu).

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1. Introduction

In the last 50 years, research has highlighted that the marine environment is vastly polluted by plastic both in the form of large plastic debris (macro and mesoplastics), microplastics (<5 mm, MPs) and nanoplastics (<1 μm, NPs) (Ryan, 2015; Sanchez-Vidal et al., 2018; Napper and Thompson, 2020; Suaria et al., 2016). As such, it has been predicted that over 170 trillion plastic particles may be floating in the world's oceans (Eriksen et al., 2014; Alfaro-Núñez et al., 2021), with concentration estimated between 0.1 and 10,000 particles/m^3 (Erni--Cassola et al., 2019; Vighi et al., 2021).

MPs and NPs may be intentionally manufactured at this size scale directly by producers to fulfil specific applications in consumer products (primary sources) (Vighi et al., 2021). Secondary sources for MPs and NPs originate from the fragmentation process of larger plastic pieces. This can be due to a combined effect of photo-oxidative degradation overtime or mechanical stress (shear and abrasion) (Huang et al., 2021; Chubarenko et al., 2020; Gonçalves and Bebianno, 2021; Browne, 2007; Galgani et al., 2010; Cole et al., 2011; Law and Thompson, 2014). The quantity of MPs in the marine environment is expected to grow substantially in the next decade if no action is taken by regulatory bodies (Boucher and Friot, 2017; Suaria et al., 2020a; Vighi et al., 2021). Additionally, it has been stated that if emissions were to cease with immediate effect, the quantity of MPs in the marine environment would continue to accumulate as a consequence of the fragmentation of larger items (Suaria et al., 2020b; Napper and Thompson, 2020; Ferrero et al., 2022; MICRO, 2022).

Further, the recent outbreak of the COVID-19 pandemic has driven an additional increase in the production of plastic worldwide; particularly single-use Personal Protective Equipment (PPE) (Silva et al., 2021). According to a report by Shams et al. (2021), delivery of personal protective equipment has increased from 5.5 million pieces to 50.4 million between June and July 2020. Additionally, the production of non-woven polypropylene fabrics, which are applied in the filter layers applied to facial masks, has increased by 300% between 2019 and 2020 (Uddin et al., 2022). It has been stated that COVID-19 PPE has increased the quantity of plastic waste discharged in the environment (Shruti et al., 2020); for example, it has been estimated that 1.56 million face masks entered the oceans in 2020 (Peng et al., 2021). Once dispersed in the marine environment, this material may fragment, spreading in the form of secondary MPs and NPs (Saliu et al., 2021).

Considering the effect of plastic pollution, a variety of marine habitats have been found to be impacted by micro and nano plastics (Soares et al., 2020; Gorokhova, 2015); however, coral reefs are of particular concern due to marine biodiversity variety, endangered species, and the importance of the ecosystem services provided (Axworthy and Padilla-Gamiño, 2019; Reichert et al., 2019; John et al., 2021; Montano et al., 2020; Isa et al., 2022). In coral reefs, surface water concentrations of microplastics have been found between 2.4 and 15.9 items/L (Ding et al., 2019; Rotjan et al., 2019; Tang et al., 2021; Huang et al., 2021). Additionally, microplastics have been found attached to the mucus and within the tissues of different wild-collected coral species (Martin et al., 2019; Vencato et al., 2021; Raguso et al., 2022). Furthermore, in the context of the global warming and global coral bleaching event (the process in which stressed corals expel their symbionts, causing them to turn white), it is questioned whether the presence of MPs and NPs may act as additional stressors on coral health and may impair their function by possible synergy with other environmental stressors (Saliu et al., 2022, Huang et al., 2021; Corinaldesi et al., 2021; Raguso et al., 2022).

Currently, there are only two laboratory-based exposure studies that have researched the impact of secondary plastic particles on corals: the first study was carried out by Reichert et al. (2019) and involved PE microbeads ranging from 65 to 400 μm to the scleractian species *Pocillopora verrucosa*, *Acropora muricata*, *Porites Lutea,* and *Heliopora coerulea*. A further study was carried out by Corinaldesi et al. (2021) and assayed a mixture of particles composed by 76.6% polyethylene, 10.9% polypropylene, 7.3% polystyrene, 3.3% polyvinylchloride and 1.8% polyethylene terephthalate in the shape of fibers and beads, and with a dimension ranging from 20 to 100 μm on the coral species *Corallium rubrum*. Both these studies highlighted adverse effects caused by secondary MPs onto coral physiology such as reduced growth, change in feeding activity, and an increase of the stress at the molecular level (such as oxidative DNA damage and the alteration of the expression levels of key genes involved in the antioxidant activity, DNA repair, protein synthesis, and electron transport systems). Furthermore, Research by Marangoni et al. (2021) reported a significant increase in the oxidative stress for scleractinian (*Stilophora pistillata)* after exposure to primary polystyrene NPs, with the average dimension equal to 0.026 μm, but no mortality.

The effects from plastic particles to corals are shown to be dependent on particle size, shape, and chemical makeup (Lanctôt et al., 2020; Okubo et al., 2018), but there is limited understanding on the impact from NPs. Therefore, the aim for this research was to assay the possible ecotoxicological effects to corals from environmentally relevant concentrations of nano and microplastic particles. The coral tested was *p. flava* as it represents the second most common group of benthic animals on shallow reefs (Norström et al., 2009) and is a diverse component of coral reef communities; providing food, suitable habitat, shelter for reef dwellers, and other services that underpin ecosystem biodiversity (Steinberg et al., 2020). Additionally, the coral species has been used as a suitable model to assess impacts related to anthropogenic stressors (e.g. temperature and acidification) and to provide insights into the octocoral resilience mechanisms (Vargas et al., 2022). The experiments used secondary NPs polypropylene fibres obtained by the artificial weathering of the non-woven fabrics retrieved from the filtering layer of commercially available surgical masks. It was hypothesized higher quantities of nanoplastic fibers will induce increasing stress onto *p. flava* soft corals and they will display evident morphological alteration.

2. Materials and methods

2.1. Nanofiber preparation and characterization

Secondary nanofibers were prepared by submitting polypropylene fibers from nonwoven fabrics to accelerated weathering, following the procedure described in Saliu et al. (2021). This includes recovering melt blown nonwoven polypropylene fabrics from the filtering layer placed in the middle of commercial surgical masks. The fabric was cut into approximately 5 × 5 mm pieces (290-270 mg) avoiding the point of ultrasonic sewing. The chemical identity of the fibers was confirmed by microFTIR analysis by employing a PerkinElmer Spotlight 200 Spectrum Two apparatus with MCT detector operating in transmission mode as described in Saliu et al., (2021). The material was then exposed to UV light by using a UV-A lamps (340 nm; 0,76 $W \cdot m^{-2} \cdot nm^{-1}$) at 65 °C for a total time of 180 h. For uniformity, the procedure was applied to five different pieces of nonwoven fabric retrieved from five different surgical masks (different vendors) and the nanofibers obtained from each batch were collected and placed in the same glass vial. Morphological characterization of both the pristine and UV-A treated fibers was carried out by Scen Electron Microscopy (SEM) employing an HITACHI TM3030 Plus instrument equipped with a backscattering detector. The samples were mounted on aluminium stubs using carbon tape. Fibers were analyzed at 15 kV at a magnification of 500x.

2.2. Preparation and characterization of the nanofiber stock solution

The weathered polypropylene nanofibers obtained as described in the previous section were then used to prepare the stock solution for the toxicological assay. Specifically, 30 mg of nanofibers were weighed with an analytical balance (Kern ABS ABJ) and then dispersed in 20 mL of artificial seawater (36.7 salinity and 8.12 pH) by repeated sonication using a probe sonicator (Vibra-Cell VC 505 PULSER), at 20 ± 0.1 kHz for

30 s. Subsequent dilution (to 50 mL and then to 100 mL) resulted in a cloudy solution. In order to remove any floating fibres on the solution surface or fiber aggregates formed during the dilution process, the supernatant was removed by decantation and the solution was filtered (1 μm pore size, Puradisc, Whatman) then diluted to a final volume of 1.0 L (corresponding to a concentration of 30 mg/L).

The quantity of material removed from the original solution by this purification process was assayed both by weighing the material retained in supernatant and filter and by assaying the concentration in the final solution by filtering an aliquot. Specifically, the supernatant fraction was filtered onto the same filter used for the purification of the test solution, and the filter was weighed prior and after the filtration onto an analytical balance assay. A second filter was used in the filtration of an aliquot of the final test solution. It should be highlighted that this procedure may lead to some degree of uncertainties in the determination of the final concentration applied and may result in an overestimation of the tested concentration. However, the filtration procedure is necessary to assure that only the colloidal fraction containing only the fiber aggregates in the nanosize range is applied during the test.

Dilution of the stock solution resulted in four-test solutions defined as environmental, low, medium and high concentration, plus control, that was then employed for the ecotoxicological assay: 0.001, 0.1, 1.0 and 10.0 mg/L. Specifically, the lowest nanofiber concentration used in our experiment was selected to fall within the range of the current level of nanoplastic contamination within marine environment (Lenz et al., 2016). The highest microplastic concentrations were selected to reflect an increase in concentrations by up to four times compared to current levels; predicting levels by 2030–2050.

Before running the ecotoxicological assays, an aliquot of each nanofibers solution (each concentration level) was characterised by using dynamic light scattering (DLS) to determine the hydrodynamic size of the particles and to ensure the stability of the colloidal dispersion The DLS experiments were conducted using a Malvern Zetasizer (Malvern Instruments, Malvern, UK), with a HeNe laser at a wavelength of 633 nm and a fixed scattering angle of 90°. Measurements of the size of the polypropylene secondary nanofibers (nano-PP) were collected every 15 s at 25 °C for a total time of DLS measurement of 25 min. Data were calculated considering the obtained autocorrelation function using the cumulants method of three independent experiments (Mean ± SD). An aliquot of 100 mL of the 10 mg/L solution was concentrated in a vacuum oven at 80 25 °C, dissolved in xylene, and reprecipitated to obtain a suitable surface for micro-FTIR analysis. The stability of the colloidal phase of the nanofiber solutions was assayed both visually and by DLS before running the ecotoxicological test. A 10 ml aliquot of the test solution were placed in beakers like those used with the corals in the assays. The appearance of any change in the particle distribution was monitored visually and by collecting test aliquots every 12 h. Similarly, the stability was assayed also during the ecotoxicological test collecting aliquots directly from the coral test beaker.

2.3. Sample preparation

Ecotoxicological assays were carried out by employing specimens of *Pinnigorgia flava* raised at the Genova Aquarium (Italy) dedicated to a joint research project between University of Milano-Bicocca and Acquario di Genova. 30 *P. flava* fragments of about 3 cm, 2.5 g, displaying an average of 32 (±6.8 SD) polyps per fragment, were collected with pliers from six different random colonies. The fragments were immediately fixed on supports made of two-component epoxy resin and transferred inside the acclimatization tank for 1 week. In this tank (3 × 1x0.7 m, 3100 L, composed by acrylic and glass resin), the water was delivered by a pump (Astralpool, Victoria Plus) with a 24-h flow rate of 8 m^3 h^{-1} (to ensure complete water change every about 30 min) and reinserted into the tank after passing through the filtration system. The filtration system was composed of a sand filter (Astralpool Artic, filtering particles from 0.4 to 2 mm), and a UV filter (Panaque 750 s AB 4 lamps of 40 W). A solution of 2 L of water containing the algae *Tetra selmis* (with an average concentration of 1.5 million cells/mL) and zooplankton belonging to the Phylum Rotifera (the average concentration of zooplankton is 250 individuals/mL and the average dimension is 0.5 mm) were added daily inside the tanks in order to feed the corals. Inside the tanks, the temperature was maintained at 25 °C with an irradiance of about 200 μmol photons m^{-2} s^{-1} (12 h:12 h light: dark cycle). Chemical parameters such as salinity, pH, ammonium, nitrite, and nitrate concentration were monitored before and after the treatment to assure the absence of significant variation during the assays (Further details are reported in Table S1 of supplementary).

2.4. Study design and assessment of the endpoints

After one week of acclimatization, single coral fragments (N = 1 for beaker) were transferred in 0.6 L-capacity glass beakers (N = 30) filled with 0.5 L of artificial seawater; this provided the control experiment (N = 6 replicates) and the four test toxicological assays concentrations (0.001, 0.1, 1.0 and 10.0 mg/L; N = 6 replicates for each exposure concentrations for a total of N = 18 runs). Each beaker was equipped with an air pump, to allow water motion and oxygenation. Beakers were allocated in a water bath aquarium's tank (400 L) to maintain the temperature of 25 °C. Coral fragments were randomly assigned to exposure and control treatments.

At 0, 2, 4, 6, 12, 48, and 72 h, the following endpoints were assessed: 1) mortality, 2) percentage of retracted polyps, 3) quantification of the polyps' retraction state, 4) percentage of colonies displaying the presence of mucus, 5) classification of the amount of mucus production, 6) percentage of colonies presenting bleaching, 7) quantification of bleaching amounts by semi-quantitative classification, 8) percentage of colonies presenting tissue swelling and 9) quantification of swelling amounts by semi-quantitative classification. Details of the classification adopted for each endpoint are reported in sections 2.4.1 - 2.4.5. Health parameters, requiring semi-quantitative classification, were assessed visually using a four-level scoring system. Parameters were scored on a scale of 0 (normal limits) to 3 (severely affected), with an intermediate condition evaluated by applying a 0.5 unit (thus half-scores are permitted). This scoring system was adapted from a histologically verified stress index developed for a coral health assessment that has been previously used for evaluating hydrocarbon effects on corals (Renegar et al., 2015, 2017b; Renegar and Turner, 2021). Assessments were performed by two research staff members independently; all results are reported with Standard Error. More details regarding the protocol are reported in Table s2 within supplementary information.

2.4.1. Assessment of polyps retraction

The percentage of retracted polyps was assessed by considering the number of completely closed polyps of each fragment at 0, 2, 4, 6, 24, 48 and 72 h. The polyps' retraction state was defined by classifying them into 4 main classes (with the 0.5 unit for the classification of intermediate condition): polyps that were normally extended or slightly retracted received a score of 0; polyps that were retracted and partly closed received a score of 1; fully closed polyps received a score of 2; and those with very tightly retracted polyps received a score of 3 (further information on this scoring methodology is provided in supplementary information). The last classification state (score 3) has previously been used for researching the effect of nanoplastics on *P. flava* and the percentage values of polyp retraction (Turner et al., 2021).

2.4.2. Occurrence and quantification of mucus production

The presence of mucus was monitored in all testing corals at every time point. Primarily, the production of mucus was noted if present. Secondly, the amount of mucus production was quantified according to the scoring system protocol applied by Renegar et al. (2017): since all corals continually produce a thin layer of mucus (Brown and Bythell, 2005) this condition is evaluated with a score equal to 0; corals showing

an amount of mucus greater than this normal level but localized receive a score of 1; a score of 2 is given to corals producing more significant amounts of mucus, with more visible mucus strings or thin sheets extending upward from the coral; corals producing copious amounts of mucus, in thick sheets or with pools of mucus around the base of the coral receives a score of 3. Mesenterial filament extrusion is considered possible, but we did not see it in these exposure experiments.

2.4.3. Presence and quantification of coral bleaching

The presence of coral tissue bleaching was evaluated by checking each coral at every time point compared to the color present at the beginning of exposure (t = 0), subsequently, the occurrence of tissue bleaching was noted as present or absent, and then the values were reported as a percentage in each treatment: a score of 0 was assigned to corals displaying their original coloration; corals with slight lightning on color received a score of 1; a score of 2 represents a coral that is moderately bleached; and a score of 3 indicates significant bleaching/loss of color. To quantify the tissue bleaching correctly, corals were photographed under the same lighting conditions at each time point and reviewed by three research staff members. Moreover, in order to have a further assessment of the bleaching process, corals were monitored with the Coral Health Chart, created by the University of Queensland in 2002 (Fig. 6 within supplementary information).

2.4.4. Tissue swelling

The occurrence of swelling of tissue was evaluated by checking each coral at every time point, noting the presence or absence of the process. The tissue swelling was evaluated also by a semi-quantitative classification: this attributed a score of 0 when no swelling was observed; a score of 1 for a slight localized swelling usually in the coenenchymal tissue; a score of 2 for extensive coenenchymal swelling, and a score of 3 for extreme swelling.

2.4.5. General health status

To evaluate the overall impact caused by nanoplastics on the general health status in *P. flava*, the values of the semi-quantitative classification of polyp retraction, mucus production, tissue bleaching, and tissue swelling were considered collectively to provide each *P. flava*, fragment a score ranging from 0 (healthy corals) to 12 (heavily stressed corals) as described in Renegade and Turner (2016).

2.5. Statistical analysis

Statistical analyses were performed by employing the software IBM SPSS 28. A Kruskal-Wallis test was performed in order to evaluate significant differences in the percentage of open tentacles, percentage of corals showing the production of mucus, and percentage of corals showing tissue bleaching and swelling at NPs concentrations tested (as detailed in section 2.4). This was then followed by independent-sample t-tests in order to evaluate score differences from each evaluated parameter, considering the initial and final condition at the end of the 72 h exposure time (based on the assay protocol described in section 2.4). Specifically, the Mann-Whitney *U* test was applied to evaluate the different results obtained at the different concentration and exposition time.

3. Results

3.1. Characterization of the nanofibers

Scanning Electron Micoscopy (SEM) images of the polypropylene fibers collected after the application of the accelerated ageing treatment displayed the presence of fractures and of micro and nanosized aggregates (Fig. 1). DLS analysis carried out on the solution obtained after sonication and filtration of photodegraded PP fibers indicated the presence of a polydisperse particle distribution displaying an average hydrodynamic size of 114.7 ± 8.1 nm, and a polydispersity index (PDI) of 0.431. These results are similar to those previously reported for secondary nanoparticles of PP obtained by mechanical degradation (Ren et al., 2018). Analysis of the fibers by micro-FTIR displayed the characteristic absorption bands at 2914 and 2843 cm^{-1}.

3.2. Mortality assessment

No mortality of *P. flava* specimens was observed in both the controls and various PP concentration exposures over the 72 h Time period.

3.3. Polyps retraction evaluation

The polyp's retraction assays (values of polyps retraction expressed in percentage), at the highest concentration of nanofibers (10.0 mg/L) resulted higher after 72 h. However, this difference was not statistically significant (One-way ANOVA, p = 0.48) (Fig. 2a.). Additionally, the increase in the polyp's retraction score at the end of the exposure time (72 h) among the various treatments (Fig. 2b.) was not significant (One-way ANOVA, p = 0.56). Data showed that within the same treatment, the polyp retraction score increased significantly between 4 h and 72 h in the control and at 10 mg/L (Mann-Whitney *U* test, p = 0.015 and p = 0.020).

3.4. Mucus production evaluation

The mucus production evaluation showed that at the highest concentration of nanofibers (10 mg/L) coral fragments started to show evidence of stress such as an abnormal production of mucus already after 2 h of treatment. This production of mucus persisted and increased throughout the exposure time: after 4 h of treatment, more than 80% of fragments showed the presence of this stress factor (Fig. 3a.). No production of mucus was reported in control fragments, assuring the good health status of the specimens inside each chamber. Therefore, the production of mucus is related only to the exposure of polypropylene nanofibers. The test showed also no production of mucus at the lowest concentration of nanofibers (0.001 mg/L). At the concentration of 1 mg/L fragments started to produce mucus after 4 h of treatment, reaching the maximum percentage of 50% of fragments producing mucus after 24 h of exposure. An increase in nanofibers concentration was found to have a significant increase in abnormal mucus production for *P. flava* (One-way ANOVA, p = 0.001) (Fig. 3b.). After 72 h, significant differences were found at 1 mg/L and 10 mg/L compared to control fragments (One-way ANOVA, p = 0.015, p = 0.001), and among 10 mg/L with 0.1 mg/L and 0.001 mg/L (One-way ANOVA p = 0.002, p = 0.001). Starting (4 h) and final values (72 h) of the mucus production at 1 mg/L and 10 mg/L were statistically significant (independent-sample *t*-test, p = 0.035, and p = 0.003), on the contrary, this comparison was not statistically significant regarding control fragments, 0.1 and 0.001 mg/L concentration (Mann-Whitney *U* test, p = 0.99, p = 0.99, p = 0.99).

3.5. Tissue bleaching evaluation

Tissue bleaching was observed at the highest concentrations tested. Specifically, the treatment at 10 mg/L caused evidence of tissue bleaching at the highest percentage for *P. flava* fragments; with 66.7% of coral colonies classified as bleached (Fig. 4a). Intermediate bleaching percentages were observed in 0.001, 0.1 and 1.0 mg/L PP concentrations (Fig. 4a). A significantly higher percentage of bleached specimens were reported at 4 and 72 h of exposure for both 1 and 10 mg/L concentration tests (Kruskal-Wallis, p = 0.022, and independent-sample *t*-test, p = 0.026), while no significant differences were highlighted by considering the 0.001, 0.1 mg/L test concentration and the control treatment (Mann-Whitney *U* test,p = 0.06, p = 0.2, p = 0.39, Fig. 3b). No bleached fragments were found in the control treatments.

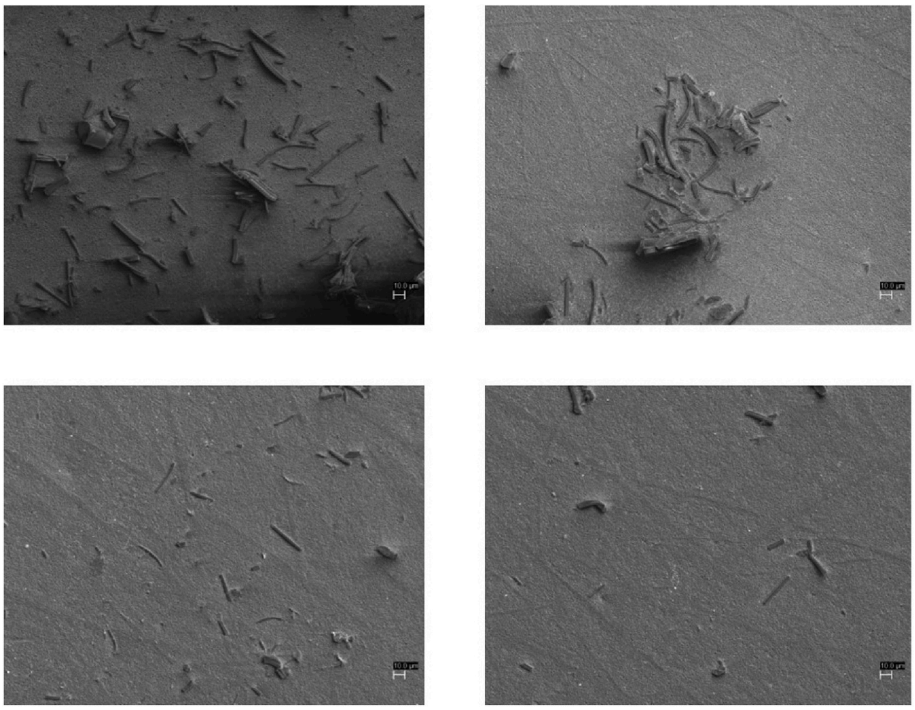

Fig. 1. SEM image of the photo-degraded polypropylene microfibers before the nanofibers stock solution preparation.

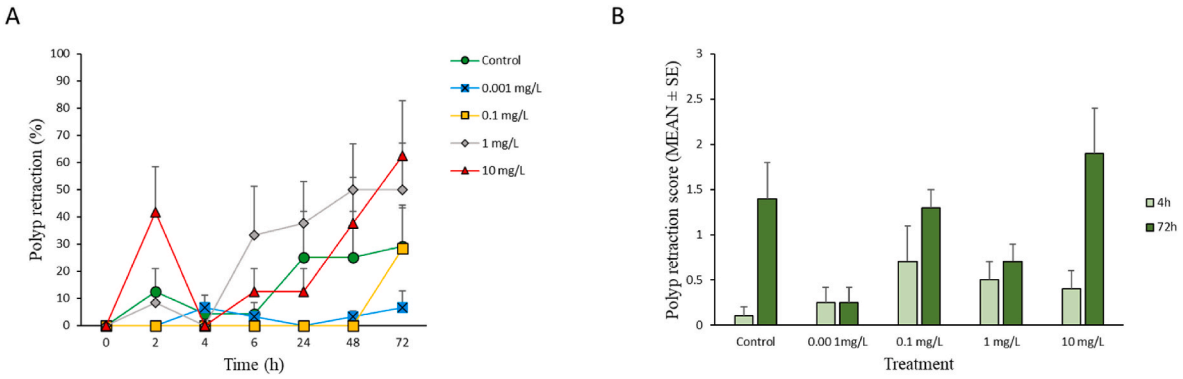

Fig. 2. Polyps' retraction in function of secondary nanoplastic concentration in *P. flava*. (a) Over time, the average value of polyps' retraction is expressed in percentage. (b) Semi-quantitative score of polyps' retraction in control and exposure batches after 4 and 72 h.

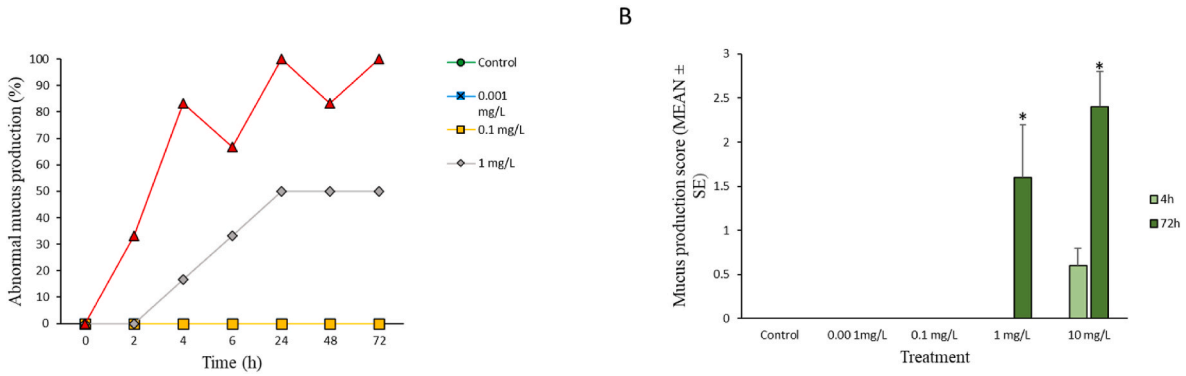

Fig. 3. Abnormal mucus production in *P. flava*. (a) Percentage of fragments showing mucus production. (b) Average values of the semi-quantitative score of mucus production according to the protocol adopted by Renegar et al. 2017) (Asterisk indicates a significant difference in respect to the control).

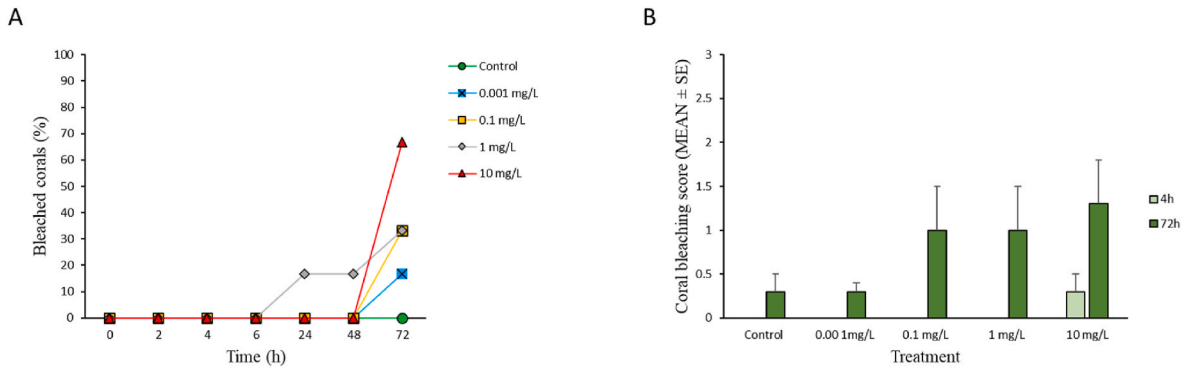

Fig. 4. Occurrence and quantification of tissue bleaching in *P..flava.* (a) Percentage of fragments showing evidence of bleaching. (b) Semi-quantitative score of coral bleaching according to the protocol adopted by Renegar et al. 2017).

3.6. Tissue swelling evaluation

No tissue swelling was observed in control, 0.001 and 0.1 mg/L treatment at any time point for the corals tested (Fig. 5a.). However, at 72 h both 1 mg/L and 10 mg/L tests showed a significant increase of tissue swelling (Kruskal-Wallis, $p = 0.015$) (Fig. 5b.). Specifically, pairwise comparisons (Kruskal-Wallis) showed a significative difference between 10 mg/L and control, 0.001 mg/L 0.1 mg/L and 1 mg/L concentration ($p = 0.003$, $p = 0.003$, $p = 0.017$, $p = 0.025$, respectively). Considering exposition time and scores of tissues swelling, a statistically significant difference between $t = 0$ and the 72 h treatment was highlighted only at 10 mg/L (Independent-sample T-test, $p = 0.049$, Fig. 5b).

3.7. General health status evaluation

The general health status was evaluated by considering the score of all the parameters described in the previous sections. Results relative to the exposure at 72 h showed that PP nanofibers had a significant negative impact on the health status of *P. flava* (One-way ANOVA, $p = 0.001$). Furthermore, Games Howell post-hoc tests showed a significant difference between 10 mg/L with control and 0.001 mg/L treatments ($p = 0.005$ and $p = 0.001$, respectively (Fig. 6.). Furthermore, after 72 h of exposure at the concentration of 10 mg/L, the impact on the general health status was significantly higher than the ones at 1 mg/L (One-way ANOVA, $p = 0.011$). According to the result at 72 h, no observed effect concentration (NOEC) is 0.1 mg/L and the lowest observed effect concentration is 1 mg/L.

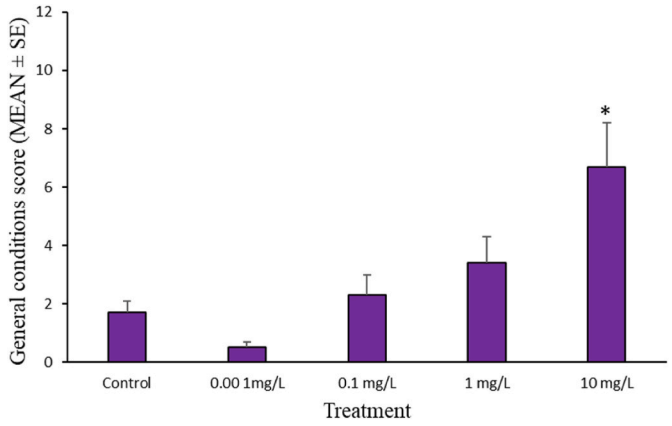

Fig. 6. General health status of *P. flava* fragments after 72 h of exposure. For each coral fragment, the general health condition was calculated by adding the semi-quantitative score of the individual parameters (polyps' retraction, mucus production, tissue bleaching, and swelling). The asterisk indicates significant differences with respect to the control.

4. Discussion

NPs research is considered still in its infancy; it has been reported that NPs occur in the environment, but analytical methods for the separation, concentration, and identification of nanoplastics are lacking (Cai et al., 2021; Bouwmeester et al., 2015; da Costa, 2018; Saliu et al., 2020).). Additionally, NPs concentrations in the marine environment are currently estimated from a model based on microplastic counts (Cózar et al., 2014; Suaria et al., 2020a). Due to the limitation of technology for extracting nanoplastic, the degradation processes that lead to the formation of NPs and the mechanism involved in the biological interaction of NPs with living organisms are not yet clear (Shen et al.,

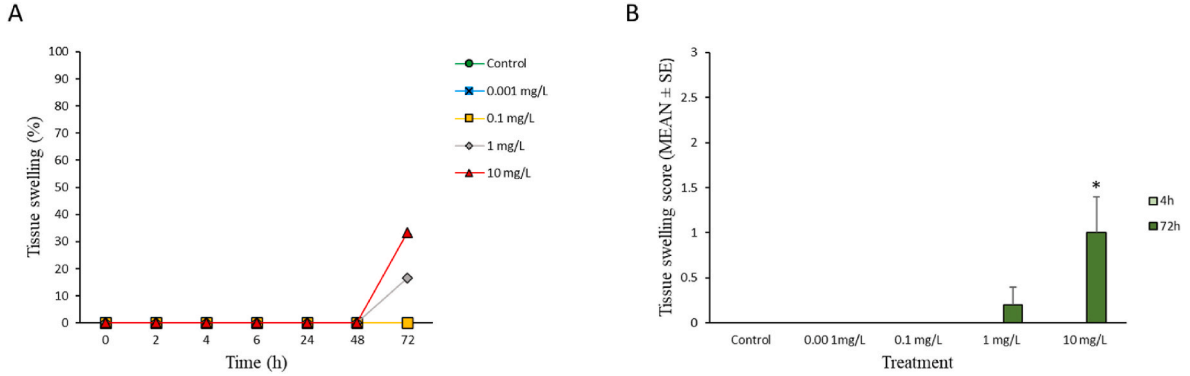

Fig. 5. Polyps' tissue swelling. (a) Percentage of polyps showing tissue swelling in the function of the time and treatment. (b) Semi-quantitative score of tissue swelling in the function of the time and treatment asterisks show significant differences in respect to the control.

2019).

To the best of our knowledge, this is the first study that investigates the potential toxic effects on corals with secondary nanosized fibers. Other studies have focused on primary NPs using particles provided by plastic vendors already manufactured at the nanoscale (Shen et al., 2019). For example: Berry et al. (2019) studied the effects of different concentrations of weathered polypropylene particles (size of about 0.22 μm) on gamete fertilization, embryo development, and larval settlement of the reef-building coral *Acropora tenuis*; Marangoni et al. (2021) assayed the effects of polystyrene (PS) NPs (20 nm size) on *Stilophora pistillata* at the concentration of 0.5 mg/L with for 4 weeks. In both cases no mortality was observed as in our trials.

Overall, our results are similar to the observation by Marangoni et al. (2022), where oxidative stress and significant bleaching were highlighted. They report that *P. flava* displayed signs of negative impact at the physiological level already after 2 h of exposure to secondary polypropylene nanofibers, and at a concentration level that is more than 3 orders of magnitude greater than the concentration estimated in the marine environment (Lenz et al., 2016; Mattsson et al., 2018).

All the endpoints assayed within our study showed a direct correlation with concentration. This is a common observation for assays involving the exposition of Alcyionacea organic pollutants; that underline the increase in mucus production with increasing exposition (Turner et al., 2016). However, the study by Turner et al., (2016) also reported that after an initial peak in the production, a decrease was then observed. Within our study we observed increasing mucus production until after the end of the treatment. This may indicate the presence of some detoxification mechanisms that are not capable of coping with nanoplastic and that should be further investigated. Moreover, since within our experiments the increase in exposure time led to a clear increase in *P. flava* stress responses, it cannot be excluded that experiments with an exposure time longer than 72 h might highlight the effect of the survivorship of corals. Therefore, exposure times greater than >72 h need to be assayed in future experiments.

Currently, there is no additional information regarding the interaction between corals and PP NPs, and thus the comparison with previous studies is limited to MPS (Allan et al., 2021). Previous studies have reported that PP MPs were found inside the mesenterial tissue within coral gut cavities, which could cause negative impacts on the health status of coral (Krishnakumar et al., 2021; Hall et al., 2015). Additionally, Corinaldesi et al., 2021 showed that fragments of the octocoral *Corallium rubrum* after a long-term (14 days) exposure to a mixture of MPs, (1000 MP particles/L at 20–100 μm) presented >50% of necrotic tissue and significant reduction in feeding activity. Furthermore, Hierl et al. (2021) observed no mortality in a five-month aquarium-based experiment carried out with specimens of four coral species (*Acropora valida, Montipora capricornis, Pocillopora damicornis, and Seriatopora hystrix*) exposed to high concentrations (ca. 0.5 g/L) of polyethylene terephthalate (PET) MPs (<500 μm).

As recently reviewed, short-term NPs exposure displayed absence of mortality in a large variety of marine organisms of different phyla (Gonçalves and Bebianno, 2021). On the other hand, some specific organisms displayed toxicity. For instance, *Daphnia Galeata* exposed to PS nanoplastic at the concentration of 5 mg/L showed a mortality of up to 83% from all the individuals after 2 of days (Cui et al., 2017). Mortality up to 60% after 69 h of exposure was also highlighted for the freshwater cnidarian *Hydra viridissima* at the concentration of 40 mg/L of Poly (methyl-methacrylate) NPs (Venancio et al., 2021). This underlines once more how the response may vary among different phyla and different species and the importance of specie-specific studies (Piccardo et al., 2020). As additional consideration, it must be considered that secondary particles do not retain the physicochemical characteristics of the original material (e.g. they may easily change in shape, size, and surface properties) and therefore they may display a different set of potential toxic effects (Boucher and Friot, 2017; Alimi et al., 2018). For instance, the photo-aged nanofibers in our test resulted in being oxidized onto the surface and thus were relatively more polar than the original microfibres. Thus, we suggest that the current ecotoxicological assay should involve NPs obtained from accelerated weathering, as we demonstrated in this wok.

5. Conclusion

In conclusion, this study showed that 72 h exposure of *P. flava* fragments to secondary polypropylene nanofibers did not cause mortality, but a concentration greater than 1.0 mg/L was sufficient to produce significant adverse effects onto the behaviour and physiology of the coral; including the retraction of polyps, production of mucus and tissue bleaching. Concentration-dependent effects were observed with the highest concentration (10 mg/L) showing the most severe effects. Considering all the behavioural and physiological parameters surveyed in the study, NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect concentration) on *P. flava* fragments after 72 h of exposure resulted from 0.1 mg/L and 1 mg/L, respectively. These concentrations are higher than those currently found in coral reefs but underline how plastic nanofibers could be considered a potential threat within these habitats. Based on precautionary principles and considering the predicted increase of plastic pollution in the next years, this study highlights that mitigating plastic release into aquatic environments is of utmost importance to conserve marine biodiversity. Furthermore, the knowledge gaps regarding NPs concentrations in marine ecosystems pose challenges in the development of assays that properly mimic the current environmental level of exposition. Subsequently, the accelerated weathering setup employed in this study for the preparation of the secondary nanofibers and their testing may represent a reference model for future ecotoxicity assays onto different marine organisms.

Credit authors statement

Valerio Isa: *Investigation; toxicological assays, Writing – original draft,* Alessandro Becchi: *Investigation-Accelerated weathering experiments,* Francesco Saliu: *Conceptualization; Investigation; Data curation; Writing – original draft,* Immogen Napper: Writing-revised draft, Silvia Lavorano: Funding *acquisition; Data curation; Project administration,* Paolo Ubaldi: *Investigation; Resources,* Paolo Galli: Funding *acquisition; Project administration*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.138509.

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