Glass reinforced plastic (GRP) a new emerging contaminant - first evidence of GRP impact on aquatic organisms

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Abstract

Plastics and synthetic materials are polluting the world’s oceans. In this study we exposed juvenile mussels, *Mytilus edulis*, to glass reinforced plastic (GRP) dust, under laboratory conditions. The study ran for a period of 7 days, to test for the morphological and potential physiological impacts of GRP. Infrared spectroscopy has revealed that the GRP resin material is poly diallyl phthalate. In mussels, particulate glass and plastics were detected in the digestive tubules and gills, with a suite of inflammatory features observed in all examined organs. In parallel, we observed the effect of powdered GRP on swimming behaviour and survival of water fleas, *Daphnia magna*. Polymer particles and fibreglass adhered to the filament hairs on appendages, including the caudal spine, in exposed organisms. Most importantly, swimming impairment and sinking of the animals were recorded shortly after exposure. The potential implications for severe localized impact of GRP on aquatic environment are discussed.

Keywords: glass reinforced plastic, mussels, inflammatory reaction, swimming impairment

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This work was supported by UKRI through the University of Brighton Ignite award to Dr. Corina Ciocan.

1. Introduction

Glass reinforced polymer (GRP) is a thermoset polymer-based composite that dominates the global boat market. As of 2011, GRP accounted for more than 80% of overall marine composites demand (Dokos, L. 2013). GRP was created in the 1930s and made commercially available for boat production from the 1950s. Hulls for small boats, produced by hand lay-up, were one of the first applications of fibreglass. Nowadays, all types of boats including rigid inflatable boats (RIBs), large

1 multihulls, canoes, warships and fast catamarans are using this versatile material (Marsh,
2 2006). Most of the GRP boats still survive to this day. However, a growing number of end-of-
3 life vessels, no longer financially viable, have substantially limited options for their disposal,
4 bar landfill. Recent studies by Rees et al, 2014 and Turner et al, 2016 highlighted the
5 increasing environmental impact of the abandoned boats in UK estuaries. The authors
6 reported that abandoned boats are a common site around UK coasts, apparently due to the
7 lack of any clear and enforceable regulation. The potential environmental impacts of such
8 decaying structures range from navigational hazard, crushing and smothering benthic
9 communities, to contamination of surrounding areas.

10 A recent report published by the International Maritime Organization (IMO) in 2019
11 recognizes that numerous bodies (user groups, government, industry) have undertaken
12 studies to consider disposal options of GRP boats. A fully viable market for the material is
13 currently lacking, as the price for recycled fibreglass is too low to promote a recycling
14 industry. Therefore, the sole legal option of disposal is landfill, which is restricted in some
15 countries, leading to disposal at sea becoming more and more common (IMO report, 2019).
16 The damage to mangrove, seagrass and coral habitats from abandoned vessels have been
17 recently investigated (Koch et al 2009, Graham and Nash, 2013) and although the effects
18 have been recorded on a relatively localised basis, the cumulative effect from the practice
19 may increase as disposal of end-of-life vessels is predicted to increase.

20 The chemical and biological effects of GRP vessel disposal at sea are yet to be revealed.
21 There are several contaminants associated with abandoned vessels in benthic / pelagic
22 ecosystems, e.g. hydrocarbons, heavy metals, flame retardants. It is reasonable to assume
23 that GRP can break down to microplastics and fibre glass; moreover, microparticles can be
24 released when the boats are crushed, dismantled or just repaired (IMO report 2019). In
25 theory, the GRP fragments have higher density than sea water and will tend to concentrate
26 nearshore, close to boatyards or where vessels are abandoned, disposed of or wrecked
27 (GESAMP 2015).

28 Fiberglass has similar chemical and physical properties to asbestos: minerals which can be
29 separated into thin, long fibres composed of Si and O, as well as other inorganic materials
30 (Galimany et al, 2009). Human exposure to both types of materials can cause fibrosis, lung
31 cancer and mesothelioma by inhalation (Maxim and Maxim, 2001).

32 Precise data on the breakdown of GRP and the scale of contamination is just not available at
33 the moment. However, as with other plastics we can assume that microparticles released
34 from GRP (fibre glass and microplastics) can potentially accumulate into the food chain by
35 zooplankton and filter feeders, and biomagnify to and in higher organisms (Wright et al,
36 2013).

37 In this study, the zooplaktonic crustacean *Daphnia magna* and benthic bivalve, the blue
38 mussel *Mytilus edulis* were exposed to powdered GRP, to test the hypothesis that GRP dust
39 will impact the physiology and health of both organisms. The GRP fragments, very similar to
40 the material resulting from cutting and sanding operations carried out in normal boatyards
41 were investigated by Raman and infrared spectroscopies. To demonstrate the effects, we

performed a histological analysis to identify the presence of GRP in mussel tissues and to screen for potential pathology. *Daphnia magna*, a well-known model species representative for zooplankton and one of the lower levels of the food chain, was analysed for signs of ingestion or/and acute immobilization following short exposure to GRP. Since the knowledge on the presence and effects of GRP in aquatic environment is extremely limited, our approach follows the precautionary principle and aims at identifying preliminary first detectable effects of the contaminant, before evaluating the risk at environmentally relevant concentrations in retrospect. This study thus provides a basis for future research on GRP impacts in aquatic ecosystems, especially in relation to possible implications for transfer up the food chain.

2. Materials and methods

2.1. IR and Raman spectroscopy

A common type of laminated GRP containing layers of fibre-rich horizons sheet sourced from a local boatyard in Chichester Harbour was manually milled cut to produce a powder (Fig. 1). This fabricated powder has been further analysed by IR and Raman spectroscopy and particle size analysis and used for comparison. Mid-infrared measurements were performed using a Perkin Elmer, Spectrum 65 spectrometer, fitted with an attenuated total reflectance (ATR) accessory employing a ZnSe crystal. Measurements through the ATR accessory requires firm contact between the sample and the ZnSe crystal. This was achieved by uniform light manual compaction. The samples were measured in the spectral range 2000-550 cm^{-1} at a resolution of 4 cm^{-1} . Each spectrum was collected from 16 scans and repeated on different subsamples to assure representative spectral information.

Raman analyses were conducted using a Perkin Elmer Raman Identichex fitted with a 785 nm diode laser and with a CCD detector, thermoelectrically cooled to -50 °C. The measurements were acquired utilizing a fibre optic probe (spot size of 100 μm at a working distance of 7.5 mm) with 100 mW laser power. Raman spectra were taken in the spectral range 2300-200 cm^{-1} at a resolution of 2 cm^{-1} . Each spectrum was collected from 8 scans for 1 second utilising 80% of laser power and repeated six times on different randomly selected subsamples to assure representative spectral information. The limit of detection for the Raman system operating on pressed powders is ca. 2 w/w % or better (Kristova et al., 2013). All analyses were conducted at 25 °C, at atmospheric pressure at the University of Brighton U. K.

Particle size analysis was performed by laser diffraction using a Malvern Mastersizer 3000 particle size analyser with a single optical measurement path. The particle sizer is equipped with series of detectors measuring the intensity of light scattered by the particles within the sample for both red and blue light wavelengths and over a wide range of angles. GRP sample was measured utilising small volume unit (100ml volume) over size range 10 nm–3.5 mm. Powdered GRP particles (5mg) were suspended in 20 ml deionised water with trace

of surface active agent (Tween 20, VWR) in concentration of 1 drop of Tween 20 per 2.5 litre water and sonicated for 2 minutes to improve dispersion/reduce agglomeration. This suspension was then introduced to further 80 ml deionised water with no additives inside small volume unit circulating through particle size analyser.

2.2. Mussel exposure to GRP: sample collection and exposure experiment

Blue mussels *Mytilus edulis* were collected manually in January 2020 at low tide from Hove beach, East Sussex, UK (50.823797, -0.173423). Mussels were sampled from one population only, kept on ice after collection, and transferred immediately to the laboratory. The animals were cleaned by removing the dirt and organic materials including algae and barnacles and then placed in a container with artificial seawater (Instant Ocean® Sea Salt, USA) for acclimatisation. During acclimatisation (3 days), the mussels were kept in the cold room (water temperature 5°C) and fed daily with a 500 µL dose of green algae *Tetraselmis* sp. culture suspension (ReefBoost, UK) per 5 litres of artificial seawater.

Mussels used in the exposure experiments were selected based on their size range (25 mm to 35 mm) and artificial seawater (Instant Ocean® Sea Salt, USA) was prepared to ensure a salinity of approximately 30.5 ppt. A total of 6 tanks (2 replicates per tank) were used to accommodate 60 mussels (10 mussels per tank): control, treatment 1 (0.3 g GRP per 5L of water) and treatment 2 (0.6 g GRP per 5L of water). All tanks were fitted with aerators and the water temperature, pH and oxygen content were recorded daily.

A GRP sheet was manually milled cut to produce a powder, which was collected in a small beaker. The GRP powder contained fragments of microplastics and glass within a size range of 40-4000 µm (Fig. 1 and Fig. 4).

The pre-selected quantity of GRP was added to the exposure tanks (T1 and T2 only). The artificial seawater was changed once after 72 hours and the tanks were cleaned; the GRP powder was then added to T1 and T2 and the experiment was terminated after 7 days. No mortalities were registered in any of the tanks.

2.3. Histopathology

Histological analysis was employed to determine the potential presence of GRP within mussel tissues and to examine the pathological conditions present in selected organs. Mussel tissues were processed prior to histological examination following a technique adapted from Ciocan *et al.* (2010). Approximately 1 cm cube of each gonad were fixed in neutral buffered formaldehyde (Sigma-Aldrich) in clean tubes. Prior to wax infiltration, tissues were placed into labelled cassettes and processed through series of dehydration and clearing using an automated tissue processor (Leica Biosystems, Germany). After paraffin embedding, solid blocks were sectioned using a manual microtome (Thermo Scientific, UK). Sections of 7 µm were transferred to slides and placed in a 37 °C oven overnight to ensure

the tissue fixation to slides. Histo-Clear™ (National Diagnostics™, UK) and descending concentrations of ethanol were used to remove paraffin from sections. After staining with hematoxylin (Sigma-Aldrich) and eosin (Sigma-Aldrich), deparaffinized sections were then dehydrated and mounted in DPX (CellPath, UK). Histological examination of tissues was performed using a light microscope (Leitz Wetzlar, Germany) (40x/100x), and histopathological conditions were recorded along with micrographs corresponding to each condition using GXCam Hichrome-Lite (GT Vision, UK).

2.4. Swimming impairment test

The pelagic copepod *Daphnia magna* (water flea) were procured from Blades Biological Ltd, UK. Samples were stored in the laboratory, at room temperature and used within 3 days. We selected only mature animals and in order to avoid contamination, we used glass material for handling of particles, handling and accommodating of daphnids and for exposure experiments. Experimental tanks were covered to reduce airborne contamination. *Daphnia* were maintained in RO filtered water at 20⁰ C; approximately 30 adults were selected for each experimental tank: Control, treatment 1 (0.3 g GRP per 5L of water) and treatment 2 (0.6 g GRP per 5L of water); two replicates per each tank. The tanks were aerated prior to the addition of the daphnids.

Fig. 1. Glass Reinforced Polymer. A – fragment of a GRP flat sheet, B – powder obtained through cutting, collected in a Petri dish; scale bars 1cm. C and D – GRP powder x 50 magnification.

The testing procedure was based on the OECD guideline *Daphnia* sp. (OECD, 2004). This guideline aims at identifying critical acute effect and threshold concentrations of chemicals towards daphnids. According to the guideline, the fraction of immobilised organisms is the criterion for acute adverse effects.

To explore the swimming behaviour and external adherence of microplastics on *Daphnia*, we first exposed the copepod to GRP powder for 90 minutes and then employed light microscopy to visualize the micro- fragments clumping on the carapace and appendages.

3. Results

3.1. Raman and Infrared analysis of GRP fragments

The mid-infrared spectrum of the GRP powder (Fig. 2) shows large number of peaks of different intensities and with many bands overlapped. Several strong high intensity peaks associated with carbonyl functional group at 1723 cm⁻¹ (assigned to carbonyl stretch C=O), 1256 cm⁻¹ (C-O-C), and 698 cm⁻¹ (possibly O-CO-C). The spectrum also shows numerous bands associated with presence of aromatic ring(s) i.e. stretching of C=C double bond (1600

cm⁻¹) and vibrations associated with deformations of aromatic ring(s) at 1064, 987 and 742 cm⁻¹. Other bands were assigned to stretches C-CH₃ e.g. 1452 and 1379 cm⁻¹. A comparison with a spectroscopic library (Perkin Elmer, polymer library) was also performed and provided a close profile match with poly diallyl phthalate (PDP) which is a common polyester resin (0.86 search score).

The corresponding Raman spectrum of the powder shows numbers of sharp generally high intensity bands which are assigned to PDP (Fig. 3), including the sharp high intensity band at 1000 cm⁻¹ which coincides with an identical high intensity spectral feature in the Renishaw spectral data base of polymers. However, the Raman spectrum of the powder also shows a range of generally low to very low intensity broad bands which cannot be readily ascribed to PDP (Fig. 3).

In the 200-600 cm⁻¹ region bands at 222, 414 and at 552 cm⁻¹ are clearly resolved. The later band appears multi-component in nature (Fig. 3). The origin(s) of these bands are uncertain. However, a band around *ca* 430 cm⁻¹ is consistent with a silica-based glass and is widely assigned to a symmetrical motion of bridging oxygens (BO) in the plane bisecting Si-O-Si bonds (McMillan and Piriou 1983). Although pure vitreous silica does not show a corresponding band at 223 cm⁻¹. Of the multitude of possible types of silica-based glasses (e.g., McMillan and Piriou 1983; Yadav and Singh 2015), bands have been reported at 412 cm⁻¹, 544 cm⁻¹ and 622 cm⁻¹ from aluminosilicate glasses in conjunction with a band at 222 cm⁻¹ (Yadav and Singh 2015). In which the three high wave number bands are assigned to bending vibrations of the bridging oxygen (BO) bonds of [SiO₄], whereas the band at 222 cm⁻¹ is assigned to the vibrations related to the Na⁺ cation (Yadav and Singh 2015). The same authors also report a band near 779cm⁻¹ caused by the Si - O - Si network and [AlO₄] units. The Raman spectrum of the powder shows a similar low intensity broad band in the same spectral vicinity, and a second low intensity band at *ca* 840 cm⁻¹ (Fig. 3). The origin of this band is uncertain but bands in the range of *ca* 820 cm⁻¹ have been reported with slight wavenumber variation for specific aluminosilicate glass types (Yadav and Singh 2015). Likewise, low intensity broad bands at *ca* 1074 cm⁻¹ and 1186 cm⁻¹ could be assigned to Si-O stretching vibrations of [SiO₄] tetrahedra (e.g., Yadav and Singh 2015).

The experimental powder also shows a prominent broad intensity high at *ca* 1868 cm⁻¹. The origin of this spectral feature is unclear, it does coincide with an infrared active band assigned to a ν''_{asym} Si-O-Si + ν'_{sym} Si-O-Si combination mode in an [SiO₂] based glass matrix (Efimov and Poggareva 2006). Accepting this it also follows that a variety of second order combinations originating from the silicate glass may also contribute to the 'intensity high' apparent background in the 1450-1650 cm⁻¹ region. Hence available evidence suggests that an aluminosilicate glass (presumably in the form of the fibre content of the GRP) is present within the PDP binding resin.

Fig. 2. Mid infrared spectrum of powdered GRP. Band assignments for some major poly diallyl phthalate (PDP) peaks are highlighted. Mid-infrared measurements were performed using a Perkin Elmer, Spectrum 65 spectrometer, samples were measured in the spectral range 2000-550 cm^{-1} at a resolution of 4 cm^{-1} .

Fig. 3. Raman spectrum of powdered GRP. poly diallyl phthalate (PDP) peaks are highlighted alongside wavenumber values listed in the Perkin Elmer, polymer library. Bands not assigned to PDP are discussed in the text. Raman analyses were conducted using a Perkin Elmer Raman Identicheck, and the Raman spectra were taken in the spectral range 2300-200 cm^{-1} at a resolution of 2 cm^{-1} .

3.2. Particle size analysis

Particle size analysis of the fabricated powdered GRP material indicates a measured mode grain size of 40 μm (by laser diffraction). The measured sample shows a polydisperse size distribution profile consistent with microscopic evidence and possible agglomerations of particle aggregates (Fig. 4). Three subsamples were measured, average $\text{dv}(50)$ calculated as 58.2 μm (SD 8.9 μm) and average $\text{D}[3,2]$ 40.5 μm (SD 4.3 μm).

Fig. 4. Particle size distribution of fabricated GRP powder measured by laser diffraction.

3.3. Mussel Histopathology

Animals in the control group did not express any of the pathologies (Fig. 5). The micrographs of histopathological conditions recorded in the observation are shown in Figure 6. Figures A and B show a section of a digestive gland with particulate matter accumulated in the stomach. A small shard can be seen in figure 6C, embedded in the connective tissue, in the mantle. Inflammatory reactions were detected in more than 60% of animals exposed to GRP, mostly in the gills and gonad tissues (Fig.6 D,E,F). Almost a third of exposed mussels presented excess lipid accumulation in the mantle and gills. No differences in the frequency of the recorded pathologies were observed between the two treatments (T1 and T2).

Fig. 5. The occurrence of histopathological conditions observed in the gonad tissue of mussels exposed to GRP for 7 days (n=10). The treatments were as follows: Control, T1 (0.3 g/5L), T2 (0.6 g/5L).

Fig. 6. Histopathological conditions in mussel tissues after exposure to GRP powder. Sections of 7 μm , stained with hematoxylin and eosin. (A,B) digestive gland, fragments of GRP particles accumulated in the stomach; (C) fiberglass shard embedded in the connective tissue in the mantle; (D) inflammation of the gills, hemocytes invading the gill filaments; (E,F) egg follicles, female gonad, inflammation of the gonad; Arrows point to each pathological condition. Magnification X 100 (A,D,E,F), X 400 (B,C).

3.4. Swimming impairment test

Light microscopy analysis confirmed that microplastics and fibreglass adhere to the external appendages of the zooplankton: small polymer fragments (less than 50 μm) were clumped around the caudal spine and carapace segments of live copepods (Fig.7). Those fragments contributed to a considerable increase of the organism weight and therefore their sinking rate, resulting in the sinking of 100% of the animals in the exposure groups, after only 90 min. No ingestion of the GRP particles has been detected after the short exposure experiment.

Fig. 7. Microplastic of different sizes adhere to zooplankton. A – microplastics trapped between the hairs of caudal spine; size bar 50 μm . B – Microplastics and glass fibres surrounding Daphnia, with small fragments trapped on the carapace; size bar 500 μm . C - microplastics clumped around the head and the compound eye.; size bar 100 μm .

4. Discussion

Our laboratory toxicity tests with *D. magna* and *M. edulis* exposed to GRP dust for a short period of time revealed morphological and physiological damage in both species, ranging from inflammatory reaction to swimming impairment.

Recently, plastic particles have been shown to accumulate in various aquatic species, such as zooplankton (Desforages et al, 2015) and filter feeders (Van Cauwenberghe *et al.*, 2015), and therefore bioaccumulate and bio magnify in fish (Wright et al, 2013), or pass directly to larger planktonic feeding organisms such as basking shark and whales (Fossi *et al.*, 2014).

GRP powder is easily released during cutting or sanding of the fibre glass sheets from boats or other structures (tubing, pontoons, GRP made outdoor furniture) and contains both microplastics and glass fibres (Fig.1).

Infrared spectroscopy has revealed that the GRP resin material is poly diallyl phthalate. However any peaks for glass fibres were obscured by the numerous peaks of the PDP or were not visible in infrared spectra. Raman spectra showed lower signal to noise ratio and was less suitable for resin identification, however it was possible to confirm PDP material. Moreover Raman spectra showed some peaks associated with glass fibres identified as silica type glass.

PDP is a material commonly used in boat industry; it is deemed inert and resistant to moisture thanks to its polymerization, however, its monomer counterpart is considered an aquatic hazard (Ge et al, 2007). Chemical stability and inertness of the secondary PDP microplastics needs to be further investigated.

The noxious effects of fiberglass ingestion in organisms are poorly understood. To the best of our knowledge, the only study to reveal the uptake of glass fibres by mussels (*Mytilus galloprovincialis* L.) was published more than 10 years ago by Galimany et al., (2009). The authors recommended further research into prey-predator relationships, along with possible implications for human health and the health of the organisms themselves.

Our results show clear uptake of the material, both plastic and fibre glass by the blue mussels exposed for just 7 days to a small quantity of GRP powder (Fig. 6 A ,B). The impressive quantity of material accumulated in the digestive gland raises concerns related to the ability of the animals to further process normal food. Several recent studies have suggested that microplastics ingestion leads to blockage of the intestinal tract and later on to death through malnutrition and starvation, in a variety of species (Duncan et al, 2019; Deudero and Alomar, 2015). In a study by Gonzales-Soto et al. 2019, large microplastics were observed in the lumen of stomach and digestive tubules of exposed mussels (*Mytilus galloprovincialis*), but were also occasionally found also within the epithelial cells. The authors concluded that at the whole organism level, a hormetic effect is evident, as a potential compensatory mechanism, with exposed mussels significantly increasing their food absorption efficiency in order to make up for the energy expended dealing with stress. No data regarding environmental contamination with GRP has been published to date, however up to 7000 pieces of glass fibres per Kg flesh, have been identified in the digestive gland of oysters (*Ostrea edulis*) and mussels (*Mytilus edulis*), collected from a natural population, close to an active boatyard (Ciocan, unpublished results).

An additional concern regarding the aquatic contamination with GRP has been demonstrated *in vitro* by the adherence of GRP microparticles to the exoskeleton of *Daphnia magna* and the severe impact on their swimming capacity. Daphnids in the treatment tanks came into direct contact with the particles mainly by swimming to the water surface. Shortly after the start of the experiment, it became obvious that in the treatment groups, some particles would attach to the carapax and appendices, which

1 impeded swimming for most of the individuals. Same results were reported in the case of
2 surface coating by PS-beads (Cole et al., 2013) or nanoparticles (Dabrunz et al., 2011)
3 experimental exposures. In their natural environment, daphnids move down- and upward
4 in the water column, sometimes being able to free themselves from the particulate
5 contaminants (Rehse et al, 2016). However, because of the very limited water depth under
6 laboratory conditions, we registered a complete swimming incapacity in 100% of the water
7 fleas in our exposed tanks, after only 90min.
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10 Of further concern is the trophic-transfer: microplastics, fibre glass (and contaminants
11 released from them) within lower-trophic, keystone organisms such as *Daphnia* may result
12 in the trophic-transfer of these contaminants up the food-chain, with the potential for
13 bioaccumulation and therefore severe consequences for higher trophic levels. All our
14 copepods from the treatment group sank after the 90 min exposures. They were coated in
15 microplastics and fibre glass, presumably because of hydrophobic- or static-attractions
16 between the microfragments and organic material (Fig.7).
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18 In live organisms, we also found that microplastics were becoming trapped between the
19 external appendages and carapace segments; very small microplastics (less than 50 µm)
20 became lodged between the filamental hairs of the dorsal spine and the fragments of the
21 carapace. Microscopical examination revealed that the additional materials stuck on the
22 exoskeleton may have had repercussions for locomotion and swimming behaviour in
23 general, therefore limiting the ability of the organisms to detect prey, feed, reproduce, and
24 evade predators, in the natural environment.
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27 The term ‘mineral fibre’ evokes similarities with naturally occurring fibres (asbestos,
28 erionite), which are the cause of various benign and malignant diseases (de Vuyst et al.,
29 1995).
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32 More recent research, such as Abbate et al. (2006), demonstrates that exposure to
33 fiberglass contributes to the development of pathologic alterations of the respiratory
34 apparatus, for which the etiopathogenesis and evolution are still unclear. The authors
35 suggest that fibreglass – independent of the environmental concentration – causes an
36 alteration of the cellular and enzymatic components of the deep lung, in working men
37 employed in different GRP processing operations and exposed to production dusts.
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39 These studies may be relevant when it comes to disposing of end-of-life boats via extrusion;
40 the dust produced in/near the marine environment, for example during dry dock boat
41 repair, can pose very real risk of entering the water column and then the food web.
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51 5. Conclusions

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53 To our knowledge, this is the first report of the GRP spectroscopy fingerprint and exposure
54 induced adverse effects on aquatic organisms. Our findings suggest that mussels and water
55 fleas are affected by the GRP dust and they are potentially able to introduce this new
56 contaminant in the food web. IR spectroscopy has revealed that the GRP resin material is
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poly diallyl phthalate. Phtalates have been acknowledged as potent toxicants, mainly associated with microplastics pollution. Further research should focus on the fate and effects of GRP in the aquatic environment and possible implications for human health.

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Fig. 1. Glass Reinforced Polymer. A – fragment of a GRP flat sheet, B – powder obtained through cutting, collected in a Petri dish; scale bars 1cm. C and D – GRP powder x 50 magnification.

Fig. 2. Mid infrared spectrum of powdered GRP. Band assignments for some major poly diallyl phthalate (PDP) peaks are highlighted. Mid-infrared measurements were performed using a Perkin Elmer, Spectrum 65 spectrometer, samples were measured in the spectral range 2000-550 cm^{-1} at a resolution of 4 cm^{-1} .

Fig. 3. Raman spectrum of powdered GRP. poly diallyl phthalate (PDP) peaks are highlighted alongside wavenumber values listed in the Perkin Elmer, polymer library. Bands not assigned to PDP are discussed in the text. Raman analyses were conducted using a Perkin Elmer Raman Identichack, and the Raman spectra were taken in the spectral range 2300-200 cm^{-1} at a resolution of 2 cm^{-1} .

Fig. 4. Particle size distribution of fabricated GRP powder measured by laser diffraction.

Fig. 5. The occurrence of histopathological conditions observed in the gonad tissue of mussels exposed to GRP for 7 days (n=10). The treatments were as follows: Control, T1 (0.3 g/5L), T2 (0.6 g/5L).

Fig. 6. Histopathological conditions in mussel tissues after exposure to GRP powder. Sections of 7 μm , stained with hematoxylin and eosin. (A,B) digestive gland, fragments of GRP particles accumulated in the stomach; (C) fiberglass shard embedded in the connective tissue in the mantle; (D) inflammation of the gills, hemocytes invading the gill filaments; (E,F) egg follicles, female gonad, inflammation of the gonad; Arrows point to each pathological condition. Magnification X 100 (A,D,E,F), X 400 (B,C).

Fig. 7. Microplastic of different sizes adhere to zooplankton. A – microplastics trapped between the hairs of caudal spine; size bar 50 μm . B – Microplastics and glass fibres surrounding Daphnia, with small fragments trapped on the carapace; size bar 500 μm . C - microplastics clumped around the head and the compound eye.; size bar 100 μm .

***Declaration of Interest Statement**

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CC: Conceptualization, Methodology, Supervision, Writing- Reviewing and Editing. **PK:** Investigation, Writing- Original draft preparation. **CA** and **MD:** Investigation. **LH:** Conceptualization, Methodology, Supervision, Writing.