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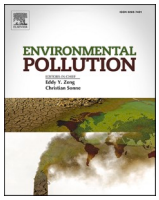

Pulse exposure to microplastics depolarizes the goldfish gill: Interactive effects of DOC and differential degradation☆

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ABSTRACT

Microplastics (MPs) are constantly degrading while moving through aquatic systems as a result of mechanical abrasion, thermal fluctuations, UV light, and chemical exposure. As such, fish may experience pulse exposures to differentially degraded plastics. This study addresses how pulse exposures, over the course of minutes, to differentially degraded microplastics alters a key ionoregulatory property of the goldfish gill. We used trans-epithelial potential (TEP) across the gills, a diffusion potential resulting from the differential permeability of cations versus anions, as a sensitive indicator of potential ionoregulatory effects. Virgin (non-degraded) MPs along with mechanically, UV, and thermally degraded plastics immediately depolarized the gills (less negative TEP), whereas chemically degraded MPs resulted in no change to TEP. To further explore the physicochemical interaction between the surface of the gill and MPs, combinations of MPs and a single source of dissolved organic carbon (DOC) were tested and revealed that the presence of DOC negated the effects of MPs at the gill regardless of whether DOC or MPs were introduced first. This study suggests that while MPs have the ability to cause ionoregulatory effects at the gill, the effects of ambient water quality, specifically the presence of DOC, are of greater influence.

1. Introduction

Microplastics, generally accepted as plastics ranging from 1 μm to 5 mm, can be introduced to aquatic environments through household and industrial effluent release, run-off, and atmospheric deposition (Blair et al., 2019; Gong and Xie, 2020; Tursi et al., 2022). MPs are further classified by their origin; primary MPs are initially manufactured to be less than 5 mm, while secondary MPs are formed through the degradation of larger plastics. In some laboratory studies, primary MPs have been artificially weathered to mimic natural degradation processes (Sun et al., 2020; Yuan et al., 2020). The state of the MPs (either primary or secondary) has been found to influence how they behave within the real world, including how a plastic may partition within the aqueous environment (Lehtiniemi et al., 2018; Sendra et al., 2021; Zeidi et al., 2023). MPs are often considered as chronic contaminants due to their persistent nature; however, water infrastructure and natural processes such as rainfall result in the pulse introduction, dispersal, and redistribution of MPs in aquatic systems (Xia et al., 2023; Carr et al., 2016; Stride et al., 2023; Kukkola et al., 2024; Hodkovicova et al., 2022). In addition to affecting the continuous redistribution of plastics in the environment, hydrological characteristics alter the degradation of MPs (Koutnik et al., 2021; Barber et al., 2006; Frère et al., 2017).

MPs undergo degradation by many processes in aquatic systems, including both biotic and abiotic mechanisms; for purposes of this study, only the latter will be considered. Mechanical degradation results from the abrasion of the plastic against environmental components such as rocks and sediment. Mechanical degradation results in the plastics fragmenting and marring (Cooper et al., 2010; Ravishankar et al., 2018). Ultra-violet (UV) degradation, often referred to more broadly as photodegradation, acts more prominently on plastics distributed closer to the water surface and results in the introduction of oxygen-containing functional groups, predicted to be through photo-oxidative processes (Mao et al., 2020; Tang et al., 2023). Thermal degradation occurs when MPs are exposed to large fluctuations in ambient temperature, and can result in alterations to the morphology and structural integrity (Luo et al., 2020).

In the same manner that the ambient environmental conditions can alter the degradation of plastics, water quality has also been found to

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contribute to the interaction of MPs with other environmental components such as dissolved organic carbon (DOC). MPs generally have a surface charge, promoting their interaction with charged or polar substances (Mammo et al., 2020). We continue to develop our understanding of how ionizable compounds such as organic matter bind to various ligands (including sediment, biota, or MPs). Water quality characteristics influence the loading of ionizable compounds to ligands (Barber et al., 2006; Fu et al., 2009; Seidensticker et al., 2018). Organic matter plays an important role in freshwater ecosystems including screening out UV radiation, altering and buffering water pH, providing temporal-spatial information to aquatic organisms, and decreasing the bioavailability of toxicants (Thomas, 1997),(Seidensticker et al., 2018). Physiologically, organic matter plays multiple roles including serving as nutrition, and promoting sodium homeostasis at fish gills (Fu et al., 2009; Wood et al., 2011). Similarly to MPs, DOC can differ in size, molecular weight, and functional groups present (Lam et al., 2007). The characteristics of organic matter determine the ability for that organic matter to bind to ligands and influence their interactions with biota (Playle et al., 1993; Chen et al., 2018).

Both MPs and organic matter interact with the gills of fish. As the vast majority of studies assessing MP-gill interactions are designed over more chronic timelines (hours, days, or weeks), our understanding of their immediate effects (within minutes) on fish gills, such as might be seen with the pulse of introduction or redistribution of plastics, remains limited. When exposed to MPs for at least a number of hours, there is evidence of altered respiratory rate (Xue et al., 2022; Xue et al., 2021; Gokul et al., 2023), increased mucus secretion at the gill (Limonta et al., 2019; Zeng et al., 2023; Zheng and Wang, 2023), and physical damage to the secondary lamellae (Limonta et al., 2019; Karami et al., 2016; Karami et al., 2017; Raza et al., 2023; Wang et al., 2022; Xing et al., 2023). It is unknown whether this damage is a result of abrasion of the gill surface by the plastic or by physicochemical interactions between the MPs and gill surface. In contrast, it is now well documented that the effects of DOC on fish gills are almost instantaneous, and depend on the physicochemical characteristics of the DOC (Wood et al., 2011; Galvez et al., 2008; Morris et al., 2021; Morris et al., 2024a).

Our recent review (Zink et al., 2024a) highlighted the current lack of information on the potential effects of MPs on the ionoregulatory functions of the gill, which is the primary organ of electrolyte homeostasis in fish. The first objective of the present study was to determine whether MPs acutely affect a key ionoregulatory parameter, the transepithelial potential (TEP) across the gills, in a model freshwater teleost, the goldfish (*Carassius auratus*). The TEP is the diffusion potential between the blood plasma and ambient water largely due to the differential permeabilities of sodium and chloride across the fish gill (Potts et al., 1984). Our second objective was to understand how the mode of degradation of microplastics, as discussed above, might affect the TEP. Our third objective was to understand possible interactions between natural organic matter and MPs in affecting TEP. It is well established that optically dark, aromatic DOCs of allochthonous origin, such as the Luther Marsh extract used here, have positive effects on ionoregulation, including marked hyperpolarization of the TEP to more negative values (Wood et al., 2011; Galvez et al., 2008; Morris et al., 2021; Duarte et al., 2018; Wood et al., 2003; Al- et al., 2013). (Seidensticker et al., 2018; Raza et al., 2023; Wang et al., 2022; Morris et al., 2021; Morris et al., 2024a; Zink et al., 2024a). A more negative TEP improves the electrochemical gradient for net sodium uptake at the gill. Due to the immediate response of TEP at the gill, our study virtually negates the confounding factor of gill abrasion present in longer exposures. The effects of MPs on gill TEP have not been previously studied.

2. Materials and methods

2.1. Experimental animals

Adult (1.1–1.7 g wet weight) goldfish (*Carassius auratus*) were purchased from a local supplier (Noah's Pet Ark, Vancouver, British Columbia, Canada). 85 total fish were obtained for all experiments discussed in this article. Experiments conformed to the guidelines of the Canada Council for Animal Care under protocol A22-0195 approved by the University of British Columbia Animal Care Committee. Fish were maintained in 20-L static tanks of aerated water with a 50% water change and approximately 2% body weight feeding of Nutrafin Max Sinking Goldfish Pellets (Hagen, Mansfield, Massachusetts, United States of America) occurring daily. The water supply to the system is piped directly from the City of Vancouver and dechlorinated, with the following composition: Na^+ ~0.09, Cl^- ~0.10, Ca^{2+} ~0.10, Mg^{2+} ~0.011, K^+ ~0.004 mmol/L, hardness ~3.3 mg $CaCO_3$/L, pH ~7.0. Fish were fasted for three days prior to the start of the experiment.

2.2. Microplastic degradation

High-density polyethylene microplastics (MPs) were utilized (MPP-620VF, MicroPowders, Inc., Tarrytown, New York, USA). The reported characteristics of this product from the manufacturer indicated a density of 0.96 g/mL at 25 °C, a mean particle size 5.0–7.0 μm, National Printing Inks Research Institute grind 2.0–3.0, and a melting point of 114–116 °C. All MPs were rinsed once in a 5% solution of Tween20® (CAS 9005-64-5) and subsequently rinsed three times in Milli-Q (EMD Millipore, Burlington, Massachusetts, USA), isolated by vacuum filtration through a 0.45 μm nitrocellulose filter (Whatman, Maidstone, UK), and dried in an oven at 60 °C to constant weight. These plastics were then degraded by one mode of four different modes of degradation, as outlined in Fig. 1.

Possible changes to the functional groups present on the microplastics were determined by Fourier-Transform Infrared Spectroscopy (FTIR; Tensor 37, Bruker, Billerica, Massachusetts, USA). Samples were run according to manufacturer specifications in the included OPUS software, entailing sixty absorbance scans of both sample and background taken with an interferogram size of 28440 at a resolution of 2 cm^{-1}. Spectra for each mode of degradation were compared with the spectra for virgin microplastics and new peaks and alterations to peak shape were interpreted manually using the Infrared Spectroscopy Absorption Table of LibreTexts Chemistry (LibreTexts Chemistry, 2014).

2.3. Measurement of transepithelial potential

Transepithelial potential (TEP) was measured by using an intraperitoneal catheter, which has been verified to give an identical result to that of a catheter in the bloodstream (Al- et al., 2013). Fish were anesthetized in tricaine methane sulfate (MS-222, CAS: 886–86–2) buffered to a neutral pH with sodium hydroxide (NaOH, CAS: 1310-73-2). Surgical insertion of the intraperitoneal catheter followed methods previously established (Wood et al., 2020; Po et al., 2021; Wood and Grosell, 2008). In short, a catheter (3.5 cm in length for this species) composed of polyethylene tubing (BD Intramedic, Franklin Lakes, New Jersey, USA) was filled with Cortland saline (Wolf, 1963) and inserted into the peritoneal cavity, then sutured in place behind the pectoral fin and at the base of the tail. Fish were left to recover in anesthetic-free water in isolated static tanks (aerated, and of the same water used in holding tanks described above) for 24 h. After recovery, fish were transferred to exposure vessels containing 200 mL of the experimental solution. The vessels were chosen to allow for fish to freely swim and change direction while not allowing for any darting or high-speed swimming. The volume of water utilized was of sufficient depth to allow for normal swimming while minimizing the capacity for vertical displacement. Fish were allowed to settle in the exposure vessel for 2 min prior to TEP measurements being taken.

To measure TEP, a high impedance voltmeter (pHm 82, Radiometer, Copenhagen, Denmark) was equipped with a pair of Ag/AgCl half-cell electrodes (World Precision Instruments, Sarasoto, FL, USA) routed to salt bridges (3M KCl (CAS 7447-40-7) in agar (CAS 9002-18-0) filling the

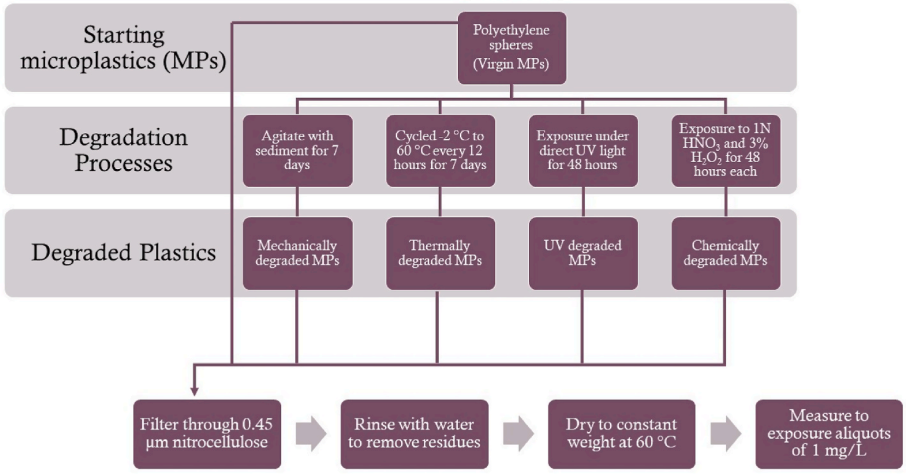

Fig. 1. Stepwise methods used to differentially degrade microplastics (by a single mode of degradation) to produce the virgin, mechanically degraded, thermally degraded, UV degraded, and chemically degraded plastics used in the study. Read as a flowchart from top to bottom, then left to right.

same polyethylene tubing as the intraperitoneal catheter described above). One electrode served as the reference electrode (submerged in ambient water of the exposure vessel) and the counter electrode was connected to the intraperitoneal catheter. Three measurements were completed within 2 min per fish, per experimental solution (Control and Microplastic, MP), totalling six data points per fish.

Series 1. Effects of differentially degraded microplastics on transepithelial potential

In series 1, each fish was randomly assigned to one of the degraded plastic types: virgin, mechanically degraded, thermally degraded, UV degraded, or chemically degraded. The three measurements for each of Control and MP were averaged after correction for the junction potential, and then used to calculate the change in TEP from Control (representing baseline TEP) and MP (representing TEP alterations caused by MP exposure). This paired design, utilizing each fish as its own control, minimizes individual variation for statistical analyses. Following TEP measurements, fish were euthanized in an overdose of neutralized MS-222. In series 1, the TEP from Control represents baseline TEP, and MP indicates TEP alterations caused by MP exposure.

Series 2. Effects of microplastics and dissolved organic matter on transepithelial potential

In series 2, in order to elicit the combined effects of MPs and organic matter on TEP, a study utilizing virgin MPs, chemically degraded MPs and dissolved organic matter from Luther Marsh (Ontario, Canada) was conducted. The characteristics and properties of the Luther Marsh DOC have been previously assessed and the concentration used in this experiment (10 mg/L DOC) was confirmed using a total organic carbon analyzer (TOC-V CSH, Shimadzu, Kyoto, Japan) (Morris et al., 2024b). The same surgical procedures for insertion of the intraperitoneal catheter and TEP measurements as above were conducted; however, rather than two experimental solutions (Control and MP, or Control and DOC), a third experimental solution (MP + DOC) was utilized, resulting in nine data points per fish.

In the first part of series 2, the addition of DOC (resulting in the MP + DOC treatment) assessed how the addition of Luther Marsh organic matter would alter the TEP changed caused by MPs when co-exposed with MPs. In the second part of series 2 (utilizing a different batch of the same-sized fish), the TEP from Control still represents baseline TEP, DOC indicates TEP alterations caused by DOC exposure, and the addition of MPs (resulting in the DOC + MP treatment) assessed how the addition of microplastics to DOC would alter the TEP change caused by DOC alone. Only virgin MPs or chemically degraded MPs were used in both experiments.

2.4. Statistical analysis

The data were analyzed using a paired statistical design to account for individual variation, meaning that the relative change in TEP was calculated as the difference from the baseline TEP of each fish. TEP was expressed as the inside voltage relative to the outside water as 0 mV. The differences among the changes in TEP between treatments were assessed using an ANOVA with Dunnett's post-hoc test. All statistical analyses and data presentation were conducted using R statistical software (v4.2.2, R Core Team, 2021).

3. Results

3.1. Microplastic degradation

The Fourier-Transform Infrared Spectroscopy (FT-IR) spectra exhibited notable differences in UV and chemically degraded MPs; however, the spectra for virgin, mechanically degraded, and thermally degraded plastics did not visibly differ (Fig. 1). The UV-treated plastics show the introduction of one small new peak not present in virgin plastics at 1710 cm^{-1}, typically associated with a carbonyl (C=O) group (Fig. 1). The chemically degraded plastics exhibited a new peak at 1380 cm^{-1}, typically associated with aldehydes (Fig. 1). The range from 2800 to 3000 cm^{-1}, containing two peaks in all other plastics associated with C-H bonds transformed into a much broader peak which when paired with large peaks ranging from 1020 to 1250 cm^{-1} is indicative of the presence of amines (Fig. 1). The same peak broadening is present in the 700-900 cm^{-1} range, indicating differences in the relative proportions of various C-H bonds, namely 1,2,3-trisubstituted and 1,2-disubstituted (Fig. 1).

Series 1. Effects of differentially degraded microplastics on transepithelial potential

Virgin (not degraded) microplastics altered TEP, causing immediate depolarization (i.e., less negative values), shifting from the control TEP of -8.8 ± 0.4 mV to -5.3 ± 0.4 mV (mean ± SEM, n = 9, Fig. 3). This trend stayed consistent for mechanical, thermal, and UV degraded plastics, shifting the TEP in a less negative direction to comparable extents (Fig. 3). The depolarization was significant ($p < 0.05$) for each of virgin, mechanical, thermal, and UV degraded microplastics. The baseline TEP (Control treatment, shown as the light bars in Fig. 3) did not differ among groups.

The change in TEP (denoted as ΔTEP), which represents the change from baseline TEP due to the presence of MPs, was calculated by subtracting the Control and MP treatments within each degradation type for

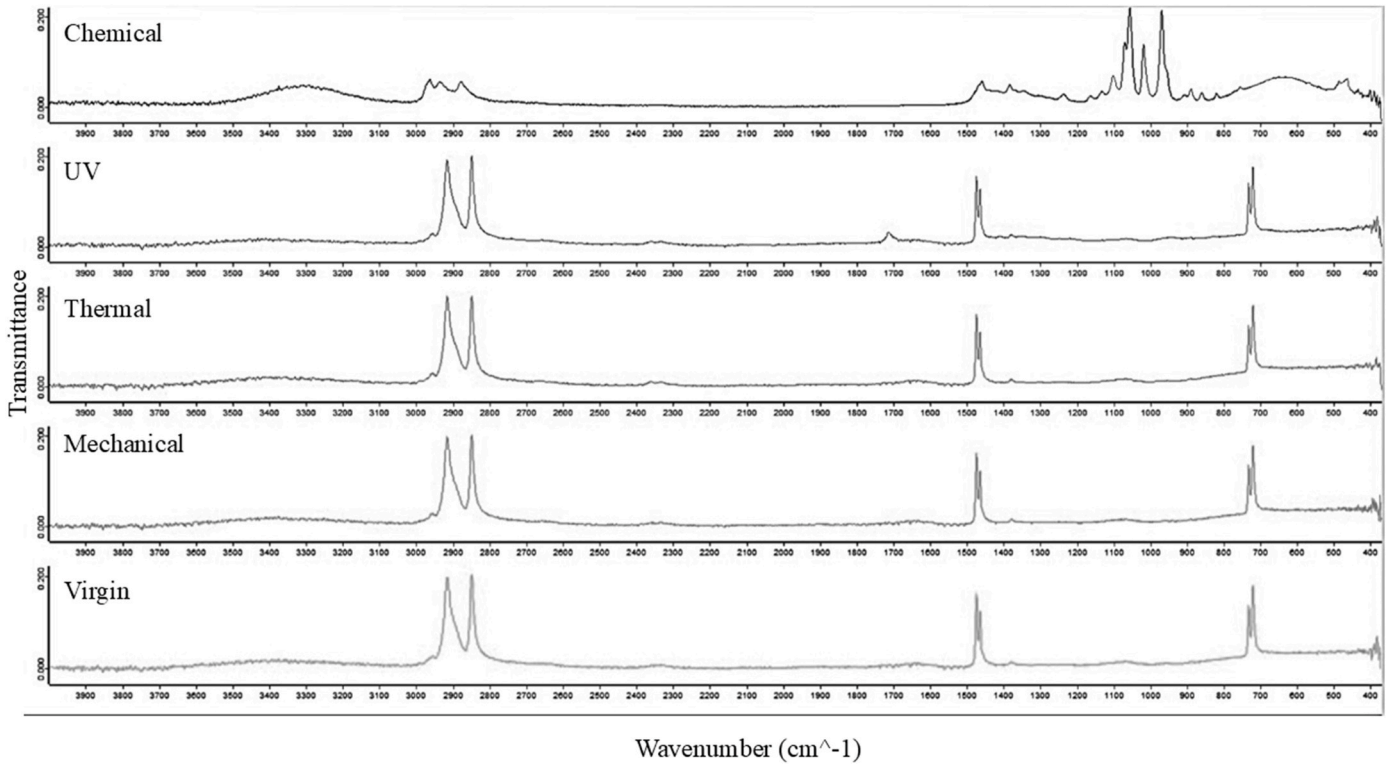

Fig. 2. Fourier-Tranform Infrared Spectroscopy spectra of each type of degraded plastic (top four panels) compared to virgin (non-degraded, bottom panel).

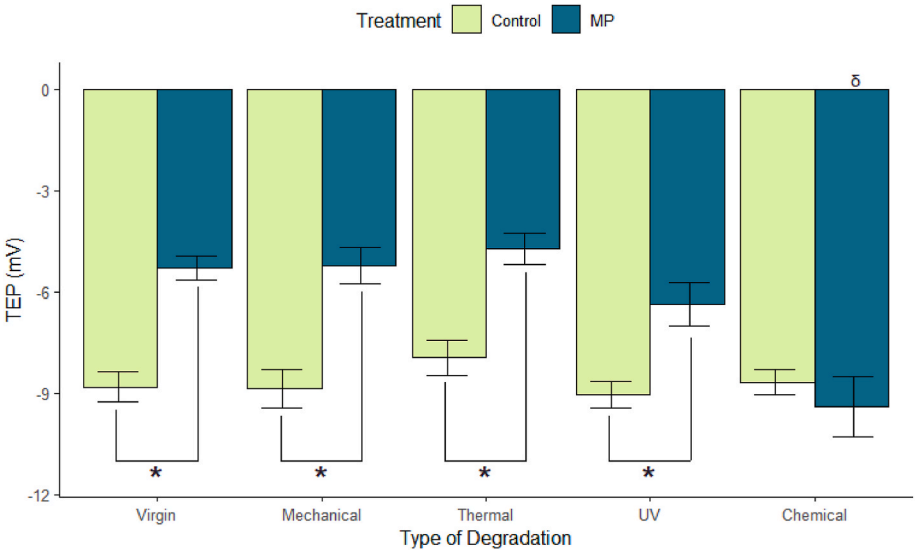

Fig. 3. In series 1, the effect of virgin (not degraded) microplastics (MP) or MPs degraded by a single mode of degradation (mechanical, thermal, UV, or chemical) on absolute transepithelial potential (TEP). Values represent mean ± 1 SEM; n = 9–11. "*" indicate statistically significant differences between control and MP of a single degradation type ($p < 0.05$ by ANOVA with a Tukey's post-hoc test). "δ" indicates significant differences in treatment across degradation types, for which no significant differences were detected across the Control treatment, while Chemical degradation for the MP treatment differed from all other degradation types.

each fish in Fig. 4 The ΔTEP resulting from exposure to virgin, mechanical, thermal, and UV degradation were not significantly different from each other, all causing depolarization of TEP, but did differ from chemically degraded MPs ($p < 0.05$, ANOVA with Tukey's post-hoc test).

Series 2. Effects of microplastics and dissolved organic matter on transepithelial potential

Following the discovery of the importance of the mode of degradation to the effect on TEP in series 1, two subsequent groups of fish were used to assess how the addition of dissolved organic carbon (DOC) would alter TEP in series 2. In the first part of series 2, the data for which are shown in Fig. 5, goldfish were exposed to MPs, then DOC addition; whereas in the second part of series 2, a different group of fish (represented in Fig. 6) experienced the same experimental conditions, except that DOC was introduced first, followed by the addition of MPs. Virgin MPs were one of the two types of MP chosen for Series 2 because in Series 1, they had exhibited the same effect on TEP as MPs degraded by mechanical, UV, and thermal treatments treatments (Figs. 3 and 4). The

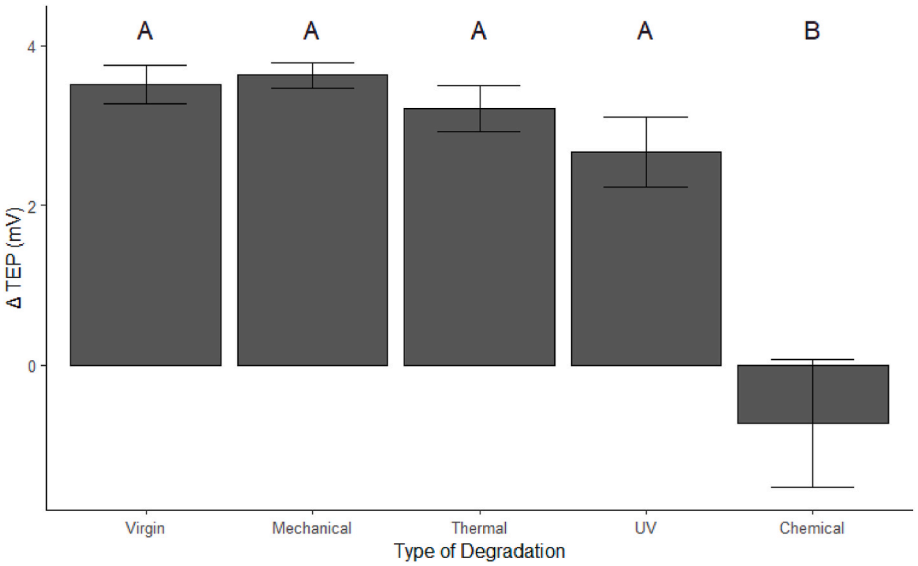

Fig. 4. In series 1, the effect of virgin (not degraded) microplastics (MP) or MPs degraded by a single mode of degradation (mechanical, thermal, UV, or chemical) on delta transepithelial potential (ΔTEP), representing net change in TEP between Control and MP Treatments shown in Fig. 2. Values represent mean ± 1 SEM; n = 9–11. Letters indicate statistically significant differences between control and MP of a single degradation type ($p < 0.05$ by ANOVA with a Tukey's post-hoc test).

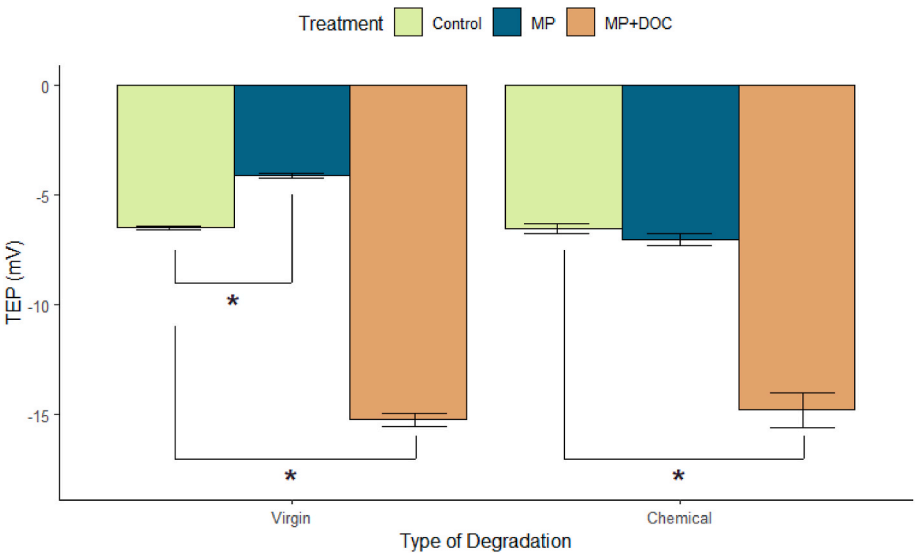

Fig. 5. In the first part of series 2, the effect of virgin (left) or chemically degraded (right) plastics (blue bars) on the baseline absolute transepithelial potential (green bars) and the effect of subsequent addition of dissolved organic carbon (DOC, orange bars). "*" indicate statistically significant differences between Control and MP and Control and MP + DOC of a single degradation type ($p < 0.05$ by ANOVA with a Dunnet's post-hoc test, n = 8). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

other type used in this test were chemically degraded MPs because in Series 1, they had differed from all other treatments in their effects on TEP. (Figs. 3 and 4).

When initially exposed to MPs fish in the first part of series 2 showed the same trends of depolarization for virgin MPs as in series 1. Again, no change in TEP for chemically degraded plastics was observed (Fig. 5). When DOC was added, resulting in co-exposure to MP and DOC (MP + DOC, orange bars in (Fig. 5), the TEP was hyperpolarized (i.e., became more negative). This did not occur with chemically degraded plastics; the baseline TEP was −6.5 ± 0.6 mV which did not significantly change following exposure to MP (7.0 ± 0.8 mV) but did show hyperpolarization with the addition of DOC to −14.8 ± 0.77 mV (mean ± SEM, n = 8).

In the second part of series 2, when initially exposed to DOC, a different group of fish showed the same trends of hyperpolarization as was observed in the first part of series 2 (Fig. 6). The hyperpolarization was less pronounced than in the first group of fish; however, the fish used in this experiment were from a different batch. When MPs were added, resulting in co-exposure to DOC and MP (DOC + MP, orange bars in (Fig. 6), the TEP was unchanged and remained hyperpolarized, regardless of the type of MP.

4. Discussion

The mode by which a plastic is degraded alters its surface composition. This study further exemplifies the need to characterize the surface of plastics prior to use in scientific experimentation or assessment (Zink et al., 2024a; Zink et al., 2023). The observed introduction and alterations to surface functional groups following UV and chemical degradation and their absence in other degradation treatments indicates that

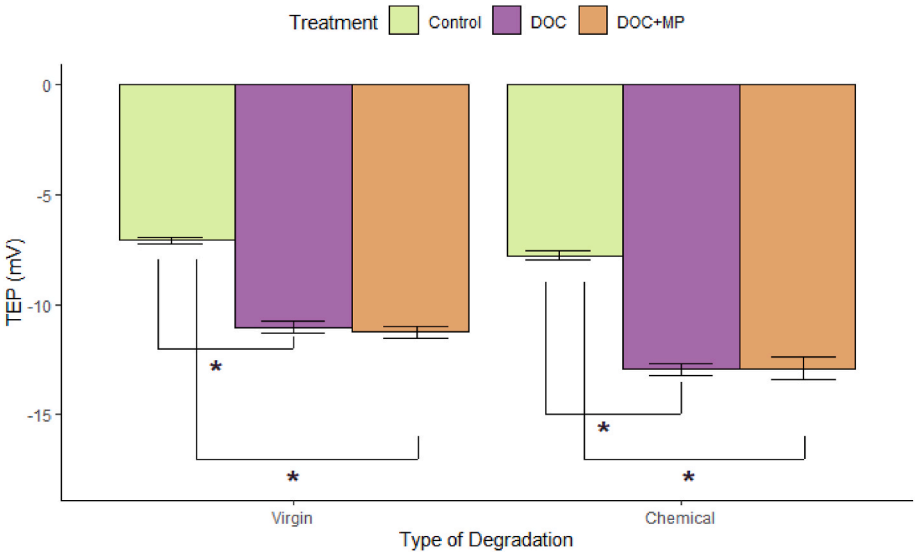

Fig. 6. In the second part of series 2, the effect of DOC (purple bars) on the baseline absolute transepithelial potential (green bars) and the effect of subsequent addition of virgin (left) or chemically degraded (right) plastics (orange bars). "*" indicate statistically significant differences between Control and DOC and Control and DOC + MP of a single degradation type ($p < 0.05$ by ANOVA with a Dunnet's post-hoc test, $n = 7–8$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

chemical reactions (by UV light or chemical compounds) primarily drive alterations in microplastic surface chemistry. The MPs used in the present study were only briefly degraded (UV and chemical exposures lasting only 48-h), much shorter than can be expected in the natural environment. It is likely that prolonged exposure to UV light or chemical compounds would result in further surface chemistry alterations (Fotopoulou et al., 2019). Chemically degraded plastics exhibited changes associated with the incorporation of nitrogen (through amine groups), oxygen incorporation as aldehydes, and alterations in carbon-hydrogen bond ratios, potentially driven by exposure to the acidic solution. Exposure to UV light resulted in the appearance of carbonyl groups, a product of photo-oxidation seen among many types of polymers (Tang et al., 2023; Tang et al., 2019).

Plastics nearer to the water surface, and therefore susceptible to greater UV-light exposure pose a greater potential for surface chemistry alterations; as such, field-collected plastics that are (or would have been previously) floating or near the surface may induce greater effects compared to deeper-residing counterparts. Plastics which are collected from acidic waters or waters containing chemical compounds likely to interact with plastics, such as downstream from water treatment facilities, are likely to yield significantly altered surface characteristics relative to their virgin counterparts.

Regardless of their position within the water column, plastics have the ability to interact with fish. This study is unique in that it negates the confounding factor of abrasive damage to the gill by exposing fish to MPs for only a matter of minutes. This type of pulse exposure would be expected during run-off, effluent discharge, and major hydrological shift events such as lake turnover. This study revealed that even brief exposures to MPs have ionoregulatory consequences, demonstrating that microplastics pose a physicochemical risk to gill surfaces rather than just a physical abrasion risk. Virgin and plastics degraded by non-chemical methods resulted in depolarization of TEP which results in ionoregulatory stress. A less negative TEP makes it more difficult for the fish to take up Na^+ and Ca^{2+} from the external water (Raza et al., 2023; Wang et al., 2022; Xing et al., 2023; Duarte et al., 2018; Wood et al., 2003; Al- et al., 2013). Interestingly, chemically degraded plastics did not alter TEP, potentially indicating that the greater degree of change to the surface chemistry (as seen on FT-IR) of the plastics during degradation affected the plastic-gill surface interactions. Further work in characterizing the physicochemical interactions of plastic and gill surfaces would help elicit a deeper understanding of the functional groups most important in mediating MP-gill interactions.

The introduction of DOC alongside MPs negated their effects on TEP, regardless of whether DOC or MPs were introduced first. This finding could indicate that the gill surface preferentially interacts with organic matter over microplastics or that the organic matter and microplastics interacted with each other, the latter being the limiting reagent. The Luther Marsh organic matter used in this study elicited a similar hyperpolarization to TEP as has been seen previously (Galvez et al., 2008; Morris et al., 2024a). Water quality characteristics have been previously observed to alter the interaction of MPs with other contaminants such as metals (Zink et al., 2024b) and this appears to also apply to MP-gill interactions when water quality, in this case organic matter presence, is altered. Future work should explore the physicochemical interactions of MPs with organic matter to elicit whether the observations of this study are due to a saturation of plastic with organic matter or if there is preferential interaction taking place at the gill surface.

5. Conclusions

Exposure to MPs, even briefly over the course of mere minutes, results in changes to TEP of the fish gill; however, it is the surface chemistry of the plastics (driven by the mode of degradation of the plastic) that determine the effect to the gill. Degradation types involving chemical reactions yielded a significantly different effect (no change to TEP) compared to virgin or plastics degraded by other means, all of which caused depolarization of TEP. The effect of plastics on TEP was negated when dissolved organic matter was present alongside the plastics. The results of this study highlight the importance of characterizing MP surface chemistry and encourages more realistic exposure scenarios as it relates to water quality, namely the importance of organic matter.

CRediT authorship contribution statement

Lauren Zink: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Carolyn Morris:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition. **Chris M. Wood:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization.

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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.

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Data availability

Data will be made available on request.

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