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Increasing microplastic exposure had minimal effects on fatty acid composition in zooplankton and yellow perch in a large, in-lake mesocosm experiment

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Abstract

Using 10 m diameter mesocosms in a Canadian boreal lake, we investigated the effects of microplastic (MP) exposure on the body weight and diet of yellow perch (*Perca flavescens*) and the fatty acid composition of yellow perch and zooplankton. We exposed the aquatic ecosystem within seven mesocosms for 10 weeks to a mixture of polyethylene, polystyrene, and polyethylene terephthalate fragments, ranging in nominal addition concentrations from 6 to 29 240 particles L^{-1} (although realized water column concentrations were lower), as well as two negative controls. Increasing MP exposure did not affect yellow perch body weight (growth) or diet, or the overall fatty acid composition of yellow perch muscle or zooplankton. Results were highly variable across mesocosms. Despite high levels of MP ingestion by yellow perch, we did not find evidence of MPs leading to food dilution or any other effect where we could anticipate impacts on food web structure.

Key words: ecotoxicology, contaminants, fish, predation, omega-3

Introduction

Microplastic (MP) pollution—defined as synthetic particles 1–5000 μm along their longest dimension—is an issue of increasing global concern, but scientists still have limited understanding of how these contaminants affect higher level biological processes, including predator-prey interactions and nutrient cycling. In aquatic systems, freshwater food webs have greater risk potential from MP pollution than their marine counterparts; many MP point sources, including sewage and stormwater outflow, empty directly into freshwater systems, which experience less dilution than marine systems, resulting in high localized concentrations (Li et al. 2018; Rochman et al. 2022). Due to higher MP concentrations, freshwater fish are more likely to ingest MP than marine fish (Covernton et al. 2021), and the highest reported concentrations of anthropogenic microparticles (including MPs) in fish digestive tracts to date were from a North American lake (Munno et al. 2021). The effects of MP exposure on aquatic organisms depend on a multitude of factors, including particle concentration, size, morphology, polymer, associated chemicals, as well as environmental conditions such as pH, temperature, salinity, and food availability (Scherer et al. 2017; Bucci et al. 2020; Piccardo et al. 2020; Lyu et al. 2022). Fish have been shown to respond to MP exposure in laboratory settings with reduced foraging behavior, mobility, and food consumption rates, as well as increased oxidative stress (Salerno et al. 2021; Hossain and Olden 2022), with critical thresholds for aquatic environments proposed in the 0.5–4100 particle/L range (Koelmans et al. 2020; Mehinto et al. 2022).

One of the leading hypotheses for how MPs might be causing harm to aquatic animals is "food dilution", whereby MPs replace natural food items, reducing assimilation of nutrients and lowering caloric and nutrient intake (Foley et al. 2018; Koelmans et al. 2020). Other proposed mechanisms of toxicity for MPs and associated chemicals include physical and chemical effects in the digestive tract or following translocation into the circulatory system and organs (Ma et al. 2021). It is likely that multiple mechanisms of toxicity occur at different life stages, depending on routes and levels of exposure. In the laboratory, fish are often able to tolerate concentrations

of MPs beyond those found in aquatic environments with limited effects on mortality, body condition, and reproductive fitness (Hossain and Olden 2022). It is, therefore, likely that MPs directly impact fish in the wild primarily in sublethal ways. Indirect effects are also possible. For example, lower trophic levels exposed to MPs may assimilate fewer nutrients due to food dilution, expend more energy due to oxidative stress, and consume lower quality prey due to MP effects on the zooplankton community. These effects could decrease energy flow to higher trophic levels, even without direct MP effects for these larger bodied animals (Hanazato 1998, 2001; Mor et al. 2022). To understand the potential effects of MPs on food web processes, it is therefore useful to look at signals relating to the flow of energy and essential nutrients such as changes in growth, shifts in prey composition, and fatty acid composition.

Fatty acids are ecologically important molecules thought to play an important role in the efficiency of energy transfer through aquatic food webs (Brett and Müller-Navarra 1997). They are essential for growth and reproduction and ecologists use them to assess nutritional quality of both predators and prey, especially via quantities of polyunsaturated fatty acids (PUFA), which are fatty acids with two or more double bonds. These include the n-6 PUFA arachidonic acid (ARA; 20:4n-6) and its precursor linoleic acid (LA; 18:2n-6), as well as the n-3 PUFA docosahexaenoic acid (DHA; 22:6n-3) and its precursors eicosapentaenoic acid (EPA; 20:5n-3) and a-linolenic acid (ALA; 18:3n-3). Mainly primary producers synthesize these PUFAs. Aquatic invertebrates can modify PUFAs and inter-convert, but fish cannot synthesize the essential fatty acids LA or ALA and have a lesser ability than invertebrates to synthesize n-6 and n-3 PUFAs with 20 or more carbon atoms and three or more double bonds. These fatty acids are known as highly unsaturated fatty acids (HUFAs), including ARA, EPA, and DHA (Parrish 2009). Aquatic fish rely on their diet for acquiring the majority of these PUFAs (Sawyer et al. 2016). Tracking PUFA quantities can thus provide information on the nutritional quality of primary producers, their consumers, and factors that might limit energy transfer to higher trophic levels (Brett and Müller-Navarra 1997). Exposure to polystyrene (PS) MPs affected the fatty acid composition of *Daphnia magna* in a laboratory experiment (Silva et al. 2017; Gonçalves et al. 2021; Parolini et al. 2022). Such MP effects on the nutritional quality of lower trophic levels have the potential to cause cascading effects in food webs.

To further our knowledge on how MPs might affect aquatic food web processes, we used an in-lake mesocosm experiment including naturally occurring plankton communities and yellow perch (*Perca flavescens*). We hypothesized that if MPs affected zooplankton community composition and (or) had negative effects on their nutritional quality, then absolute concentrations of fatty acids, including HUFAs that are important dietary nutrients for predators would decline. We further predicted that changes in zooplankton fatty acid composition could lead to changes in the fatty acid composition in muscle of predatory yellow perch, including decreasing HUFA concentrations if they are less available, or if food dilution limits their transfer. We asked the following primary questions: (1) Does increasing MP exposure decrease final body weight (as an index of mass gain) or stomach contents (diet) in yellow perch? (2) Does increasing MP exposure affect overall fatty acid composition in yellow perch and zooplankton? (3) Does increasing MP exposure negatively affect the proportional and absolute concentrations of the HUFAs DHA, EPA, and ARA in yellow perch and zooplankton, suggesting reduced trophic transfer efficiency?

Methods

Study design

Our experimental design is described in detail in Rochman et al. (2024). In brief, nine 10 m diameter mesocosms were constructed and placed in Lake 378 (L378) at the International Institute for Sustainable Development Experimental Lakes Area (IISD-ELA) in the summer of 2021. L378 is a small, oligotrophic boreal lake located in northwestern Ontario, Canada (49°41′37.88″N, 93°46′32.18″W). Each mesocosm physically separated water from the lake and consisted of a decagonal, floating, PS collar with a 2 m deep closed bottom, cylinder-shaped, nonpermeable curtain, composed of food-grade polyethylene (PE). PE pipe rings were secured to the external surface of the curtain to maintain its shape in the water. The mesocosms were anchored at a depth where they would not touch bottom then filled with ∼150 000 L unfiltered lake water, including microbes, phyto-, and zooplankton (but through mesh to exclude fish), pumped from ∼1 m depth using a trash pump (Honda Canada) connected with a fire hose.

To offset zooplankton mortality during pumping, the zooplankton communities in the mesocosms were supplemented by collecting and adding zooplankton from 15 10 m vertical hauls from the deepest point of L378 using a 0.5 m diameter net with 150 μm mesh. The mesocosms were acclimated for 5 days, after which young-of-the-year yellow perch were added to each mesocosm over an 11-day period. Collections and euthanasia at experimental end occurred under a permit from the Ontario Ministry of Natural Resources (1097798; 4 April 2021) and an animal use protocol from the University of Toronto (20012583; 16 February 2021) under the Canadian Council on Animal Care Guidelines. The yellow perch were collected from the surrounding lake using seines and trap nets. During this addition and acclimation period, fish mortalities in mesocosms were monitored using a submersible remotely operated vehicle. Mortality was initially high for trap-caught fish due to handling stress, which is why seining was subsequently employed. A similar experiment in 2022 with improved methods (seining only) and similar acclimation period resulted in low yellow perch mortality (unpublished data) supporting handling stress as the source of mortality in the current experiment. During the acclimation period, dead fish were removed from the mesocosm by free-diving, where possible, and replaced with seine-caught fish, resulting in 23–26 individuals in each mesocosm at the start of the experiment. The yellow perch ranged in size from 6.0 to 9.2 cm total length and 2.0–6.9 g weight (Table S1). These yellow perch densities reflect natural densities at the IISD-

ELA (Hayhurst et al. 2020). Following yellow perch addition, the mesocosms were acclimated for an additional 5 days.

On day 1 of the experiment, zooplankton densities in the mesocosms were lower than in the surrounding lake. Because the water in the mesocosms was separated from the rest of the lake and its nutrient inputs, and to reduce predation pressure on the zooplankton communities in mesocosms, the fish were fed a supplementary diet of fish food, thawed from frozen (Hakari Bio-Pure Mysis Shrimp, Kyorin Food Industries, Ltd., Japan). The yellow perch were fed every 3 days during the acclimation and experimental period with a daily ration of 2% (initial) body mass (~0.8% body mass by the end of the experiment)—about 0.07 g per fish per day—based on the estimated average number of surviving fish per mesocosm and according to estimated feeding rates for yellow perch (Boisclair and Leggett 1989; Hayhurst et al. 2020). This resulted in the addition of 1.26–4.80 g into each mesocosm at each feeding event over the course of the experiment.

On 2 June 2021 (day 0 of the experiment), equal proportions (by count) of PS, polyethylene terephthalate (PET), and linear low-density PE fragments were added to the treatment corrals. The MPs were added to the mesocosms in amounts that would lead to nominal concentrations of 6, 24, 100, 414, 1710, 7071, and 29 240 particles L^{-1} (sum of all polymers) if all MPs mixed homogenously into the mesocosms (which was not expected given the varying density of the particles and the complexity of transport processes) and included two control mesocosms with no added MPs. This "regression" design is an alternative to the "Analysis of variance" design, which replicates at each concentration, but results in fewer concentrations when replicates are limited. The "regression" design has been shown to be effective in ecotoxicological mesocosm experiments (Liber et al. 1992), especially for determining no effect concentrations across an exposure gradient, and when the lower end of the concentration range has more replicates (Smith and Mercante 1989), as in our design. The concentrations increased on a log-scale, representing MP concentrations found in the environment up to an order of magnitude beyond a 2050 "business as usual" projection of plastic emissions (Dubaish and Liebezeit 2013; Geyer et al. 2017). These nominal values can be considered loading concentrations, representing MPs throughout each entire mesocosm, but not at any specific point in the mesocosm. PE particles were approximately 37–1086 μm in diameter, PS particles 48–1408 μm, and PET particles 52–1408 μm. The PS was neutrally buoyant, the PET was negatively buoyant, and the PE was positively buoyant. See Rochman et al. (2024) for a full description of the plastic additives. Throughout the experiment, natural (cork) floats and hemp ropes were used to avoid additional MP contamination of the mesocosms.

Bulk zooplankton samples for fatty acids analysis were collected on 27 May (day -6, before additions), 6 July (day 34 of the experiment), and 9 August (day 68), via vertical hauls to 1.5 m with a 53 μm mesh Wisconsin plankton net with a 0.25 m diameter opening, attached to a long pole to access the center of the mesocosms. A range of one to three hauls (depending on biomass) was collected and combined into one sample per mesocosm, per timepoint. Separate zooplankton hauls were also collected and analyzed to quantify cladoceran, cyclopoid copepod, and calanoid copepod biomass for comparison with fatty acids data (see Langenfeld (2023) for details). Zooplankton composition data used for this study are from 1 June (5 days after initial zooplankton fatty acid sampling), 5 July (1 day before the midpoint fatty acid sampling), and 9 August (the same day as the endpoint fatty acid sampling).

Throughout 14–24 August (days 73–83 of the experiment), all yellow perch were collected and lethally sampled in a randomized order but with the controls at the start and end of the sampling period. Yellow perch were collected from the mesocosms using a 50′ by 6′ seine net, deployed from a small boat. The 24 particles L^{-1} treatment mesocosm collapsed during this process and so it was not possible to retrieve any of the yellow perch. A range of 4–17 yellow perch remained in each mesocosm at the end of the experiment (Table S1). Fish were weighed, had their total length measured, then were dissected. Gonads were weighed and dorsal muscle tissue from three yellow perch from each surviving mesocosm was sampled for fatty acid analysis ($N = 24$). In addition, digestive tracts were collected from one to seven yellow perch per mesocosm—depending on other sampling requirements—for stomach content diet analysis ($N = 28$). The diet samples were stored at −20 °C until analysis. All fatty acid samples were transported back to the IISD-ELA laboratory on ice then flash-frozen in liquid nitrogen. The samples were stored in a dry shipper containing liquid nitrogen until the end of the field season when samples were transported to a laboratory in Winnipeg and stored at −80 °C. The samples were later shipped on dry ice to the University of Toronto, where they were again stored at −80 °C until analysis.

Laboratory methods

The yellow perch digestive tract samples were thawed on a Petri dish, wetted, and then examined under a dissecting microscope. The stomach was separated from the lower digestive tract, opened, and the contents sorted using a microprobe. Individuals were identified to Order or superorder (Cladocera, Amphipoda, Cyclopoida, Odonata) or Family (larval or pupated Chironomidae).

Zooplankton samples were desiccated at −50 °C using a FreeZone 2.5 L benchtop freeze dry system (Labconco, Kansas City, MO, USA). Lipids were extracted from approximately 10 mg of freeze-dried zooplankton, 60 mg of yellow perch dorsal muscle, and 60 mg of fish food in 2:1 chloroform:methanol by a modified Folch method (Folch et al. 1957; Rotarescu et al. 2022). An internal standard of 40 mg docosatrienoic acid (22:3n-3) ethyl ester (Nu-Chek Prep) was included for fatty acid quantification. The resulting total lipid extracts (TLEs) for fish food and an aliquot of the zooplankton and yellow perch muscle TLEs were transesterified to fatty acid methyl esters (FAMEs) (Folch et al. 1957; Rotarescu et al. 2022), FAMEs isolated and the analyzed by gas chromatography-flame ionization detection, as previously detailed in Klievik et al. (2023).

For the fish food, three samples of each of three packages were analyzed and overall composition was, on average, 31.4% saturated fatty acids (SFAs), 19.2% monounsaturated fatty acids (MUFAs), 11.6% n-6 PUFAs, and 31.6% n-3 PUFAs (Fig.

S1). ARA accounted for 3.9% of composition, on average, EPA 9.7%, and DHA 15.6%.

Data analysis

All data analysis was performed using R v4.2.2 (R Core Team 2022). Linear mixed effects models (LMMs) were fit using the glmmTMB package (Brooks et al. 2017). Model fit was assessed using simulated residual diagnostics with the DHARMa package (Hartig 2022). Model predictions were calculated as estimated marginal means using the ggeffects package (Lüdecke 2018). Multivariate statistics were conducted using the vegan package (Oksanen et al. 2022). When using nominal MP concentration as predictor variable, we use *ln(MP concentration + 6)* for all models. This specific transformation of concentration values was used to avoid taking the log of zero values by adding the lowest nonzero value to all concentrations.

Yellow perch body weight (as a proxy for mass gain throughout the experiment) was compared among mesocosms using a linear model (LM), using the lm function, with nominal MP concentration and the number of surviving yellow perch in each mesocosm as continuous predictors. The number of survivors was used as a predictor because initial modeling with mesocosm identity as a random effect resulted in a lack of normality in simulated residuals. The variability in yellow perch body weight was found to be accounted for by number of survivors. The highest and lowest mortality occurring in the two control mesocosms and otherwise similar levels of mortality in the other mesocosms suggest primarily a mesocosm effect on mortality and supports using this as a predictor variable alongside nominal MP concentration.

The taxonomic composition of the yellow perch stomachs was visualized using non-metric multidimensional scaling (nMDS) with Bray–Curtis dissimilarity, calculated from counts by taxon, using the metaMDS function. Differences in diet according to MP exposure was assessed using permutational analysis of variance (PERMANOVA), also with Bray–Curtis dissimilarity, including fish body weight and *ln(MP concentration + 6)* as continuous predictors and mesocosm as a blocking factor, via the adonis2 function. Samples with zero counts for all taxa included in the analysis were left out of the nMDS and PERMANOVA, as they cannot be included in Bray–Curtis calculations. This excluded one of the controls from the analysis for which we only had one empty yellow perch stomach for analysis. Predictor significance was assessed using marginal effects, and treatment levels were compared with the one remaining control mesocosm via sequential one-degree-of-freedom contrasts.

Fatty acid compositional data (%) for the yellow perch and zooplankton were first reduced in each dataset to the 14 fatty acids with compositions of 1% or more. Differences according to MP exposure were assessed with canonical correspondence analysis (CCA) via the cca function. Body weight was used as a covariate, in combination with nominal MP concentration, for the yellow perch data (as opposed to total length) because it resulted in a higher proportion of constrained variance. In the zooplankton model, sampling date (days -6, 34, and 68) was included as a factor, in addition to nominal MP concentration, as well as estimated Cladocera, Cyclopoida, and Calanoida biomass ($\mu g\ L^{-1}$) for each mesocosm at the different timepoints. For all CCAs, the significance of the marginal effects of predictors was assessed using permutation testing via the anova.cca function.

The relationships between relative and absolute concentrations (mg g^{-1} tissue) of ARA, EPA, and DHA, as well as total fatty acid concentrations, with nominal MP concentration for the yellow perch and zooplankton samples were analyzed using LMM. For yellow perch, body weight and nominal MP concentration were used as fixed predictor variables and treatment as a random variable. For the zooplankton samples, just the days 34 and 68 were analyzed with timepoint as a fixed predictor and nominal MP concentration as a continuous predictor and mesocosm ID as a random variable. The models all met linear modeling assumptions according to residual diagnosis via DHARMa.

Results

Microplastic distribution in the water, zooplankton, and fish

To aid with interpretation of our findings, we provide a brief overview of data describing MP fate in the mesocosms. These data and the methods by which they were collected are covered in full in Rochman et al. (2024) and we have replicated the relevant methods from that study in the supplemental materials (ST1). The MP water column concentrations in the mesocosms were lower than the nominal concentrations added to the corral for the higher concentration additions. We measured water column MP concentrations in three of the treatment mescosms. The 29 240 particles L^{-1} nominal concentration treatment had a mean (± standard deviation) concentration of 246 (±202) particles L^{-1} across time and depths, the 414 particles L^{-1} mesocosm 13 (±8) particles L^{-1}, the 6 particles L^{-1} mesocosm 12 (±14) particles L^{-1}.Zooplankton were confirmed to ingest MPs and contained 0.06 (±0.07) particles $individual^{-1}$ in the 29 240 particles L^{-1} mesocosm, 0.07 (±0.08) particles $individual^{-1}$ in the 414 particles L^{-1} mesocosm, and 0.01 (±0.01) particles $individual^{-1}$ in the 6 particles L^{-1} mesocosm. The ingested particles were predominately PS and PE. The yellow perch digestive tracts contained 581 (±37) particles $individual^{-1}$ in the 29 240 particles L^{-1} mesocosm, 12 (±1) particles $individual^{-1}$ in the 414 particles L^{-1} mesocosm, and 1 (±0) particle $individual^{-1}$ in the 6 particles L^{-1} mesocosm. The yellow perch ingested all particle types.

Yellow perch body weight and diet

The yellow perch grew throughout the experiment, gaining an average of 5.7 g body weight; from 3.4 cm when put into the mesocosms to 9.1 cm when taken out. The LM relating yellow perch body weight at the end of the experiment (as a proxy for growth) to nominal MP concentration and number of surviving yellow perch was significant overall (adjusted $R^2 = 0.29$, $F_{2,76} = 16.76$, $p < 0.001$), but body weight was not correlated with MP concentration ($p > 0.6$), and was negatively correlated with number of surviving yellow perch in

Fig. 1. Fit from the linear model with yellow perch final body weight (*y*-axis) in relation to number of surviving yellow perch in each mesocosm (*x*-axis) and nominal microplastic (MP) concentration (colors). The points represent raw data, and the line and ribbon represent the estimated marginal mean and 95% confidence interval holding nominal MP concentration (which did not significantly correlate with body weight) at its average while varying number of surviving perch.

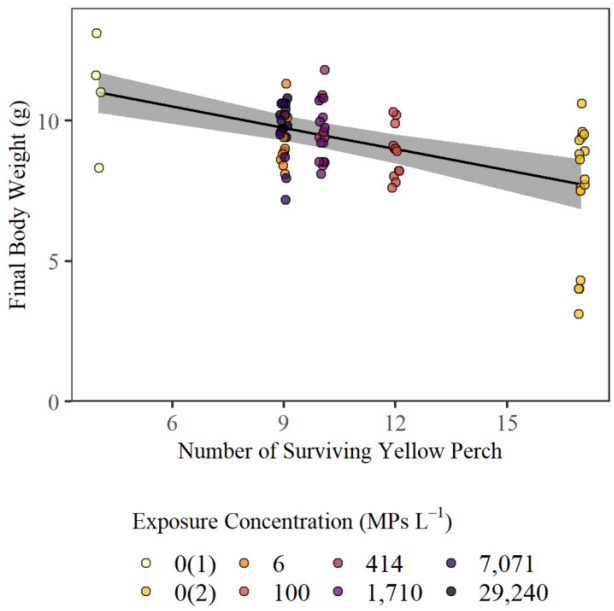
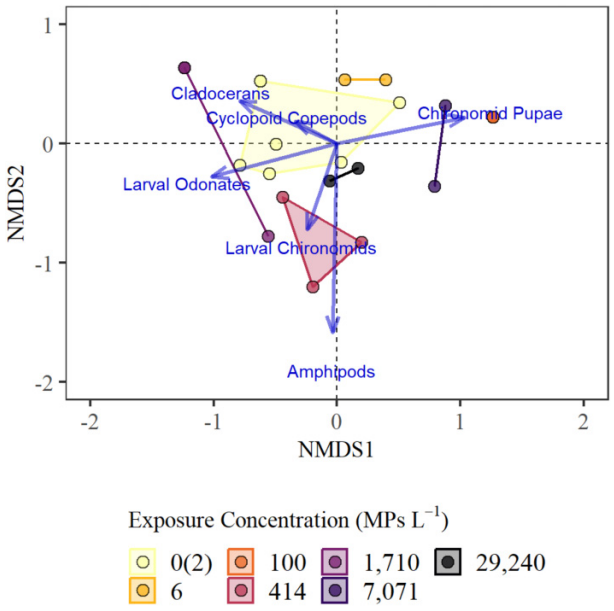


Fig. 2. nMDS plot of yellow perch diet data. Hulls connect the external points which show the position of the scores for each individual fish for each treatment/mesocosm, which is indicated by color. The blue text represents the position of the taxon scores. The first control replicate was excluded from the analysis, so the second control is labeled as "0(2)". MPs, microplastics.



each mesocosm ($p < 0.001$; Fig. 1). Across treatments, individual yellow perch contained an average of 150.6 ± 284.2 (mean ± SD) ingested invertebrates in their stomachs. The most ingested taxonomic group was Cladocera (138.2 ± 272.3 individuals), followed by Cyclopoida (9.6 ± 20.7 individuals; Figs. S2 and S3). There was no effect of either yellow perch body weight ($F_{1,21} = 0.41$, $p = 0.89$) or nominal MP concentration ($F_{1,21} = 0.86$, $p = 0.46$) on stomach content taxonomic composition according to the PERMANOVA. Inspecting the nMDS plot (Fig. 2; stress = 0.09) shows no evidence of a directional effect of MP concentration, with the highest nominal MP concentration mesocosm close to the control in multidimensional space.

Yellow perch fatty acid composition

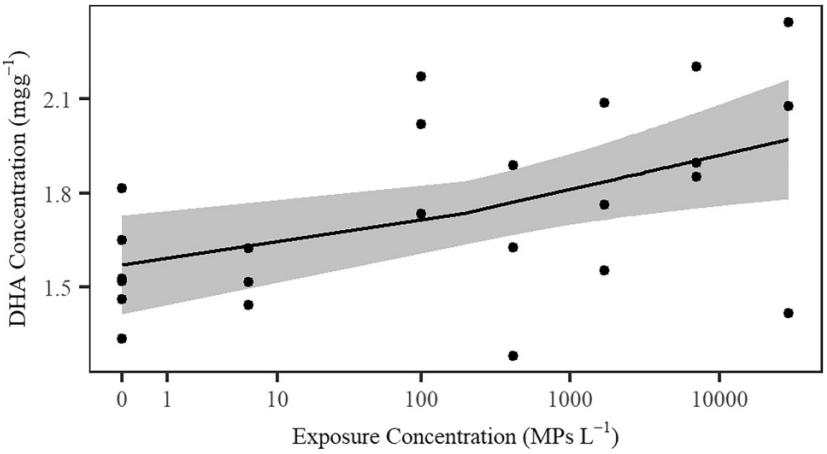
MP exposure did not affect fatty acid composition in yellow perch dorsal muscle. Across all treatments, fatty acids in yellow perch dorsal muscle were composed of 25.2 ± 0.7% (mean ± SD) SFAs, 19.4 ± 3.5% MUFAs, 16.2 ± 1.3% n-6 PUFAs, and 30.8 ± 2.7% n-3 PUFAs (Fig. S4). Individual fatty acids by proportion are shown in Fig. S5. There was no relationship between yellow perch body weight ($F_1 = 1.45$, $p = 0.23$) or nominal MP concentration ($F_1 = 0.27$, $p = 0.85$) and fatty acid relative composition according to CCA ($R^2 = 0.08$, adjusted $R^2 < 0$) and post hoc permutation tests of the marginal effects of terms. Additional analyses of the factors contributing to variation in the yellow perch muscle fatty acids are covered in the supplementary materials (ST2).

There was no effect of body weight or MP concentration on relative concentrations of either DHA ($z = -1.23$, $p = 0.22$; $z = 0.51$, $p = 0.61$, respectively) or ARA ($z = -1.11$, $p = 0.27$; $z = -0.63$, $p = 0.53$) in yellow perch dorsal muscle. There was also no effect of MPs on EPA ($z = 0.01$, $p = 0.99$), but there was a positive correlation with body weight ($z = 2.75$, $p = 0.006$), with a predicted increase of 9%–11% EPA from the smallest to largest fish (Fig. S6). In terms of absolute concentrations, there was no effect of MP concentration ($z = 1.01$, $p = 0.31$) and a weak positive effect of body weight ($z = 1.74$, $p = 0.08$) on total fatty acids. There were no effects of either body weight ($z = 1.46$, $p = 0.14$) or MP concentration ($z = 0.92$, $p = 0.36$) on ARA. There was a positive correlation between body weight and EPA ($z = 2.82$, $p = 0.005$), with a predicted increase from 1.04 to 1.79 mg g^{-1} from the smallest to largest fish (Fig. S7), but no effect of MP concentration ($z = 1.02$, $p = 0.31$). There was no effect of body weight on DHA concentration ($z = 1.35$, $p = 0.18$) but there was a positive relationship between DHA and MP concentration ($z = 2.96$, $p = 0.003$), with a predicted increase of 1.57–1.97 mg g^{-1} from the 0 to 29 240 particles L^{-1} treatments (Fig. 3).

Zooplankton fatty acid composition

There was also no effect of nominal MP concentration on absolute concentrations of zooplankton fatty acids. Comparing day 34 and day 68 of the experiment, there was no effect of MP concentration on total fatty acids ($z = 0.30$, $p = 0.76$),

Fig. 3. Relationship between concentration of DHA (mg g^{-1} tissue) in yellow perch dorsal muscle (*y*-axis) and nominal microplastic (MP) exposure concentration (*x*-axis). The points represent raw data and the line and ribbon represent estimated marginal mean and 95% confidence interval predicted from a linear mixed effects model while holding yellow perch body weight at 9.64 g.



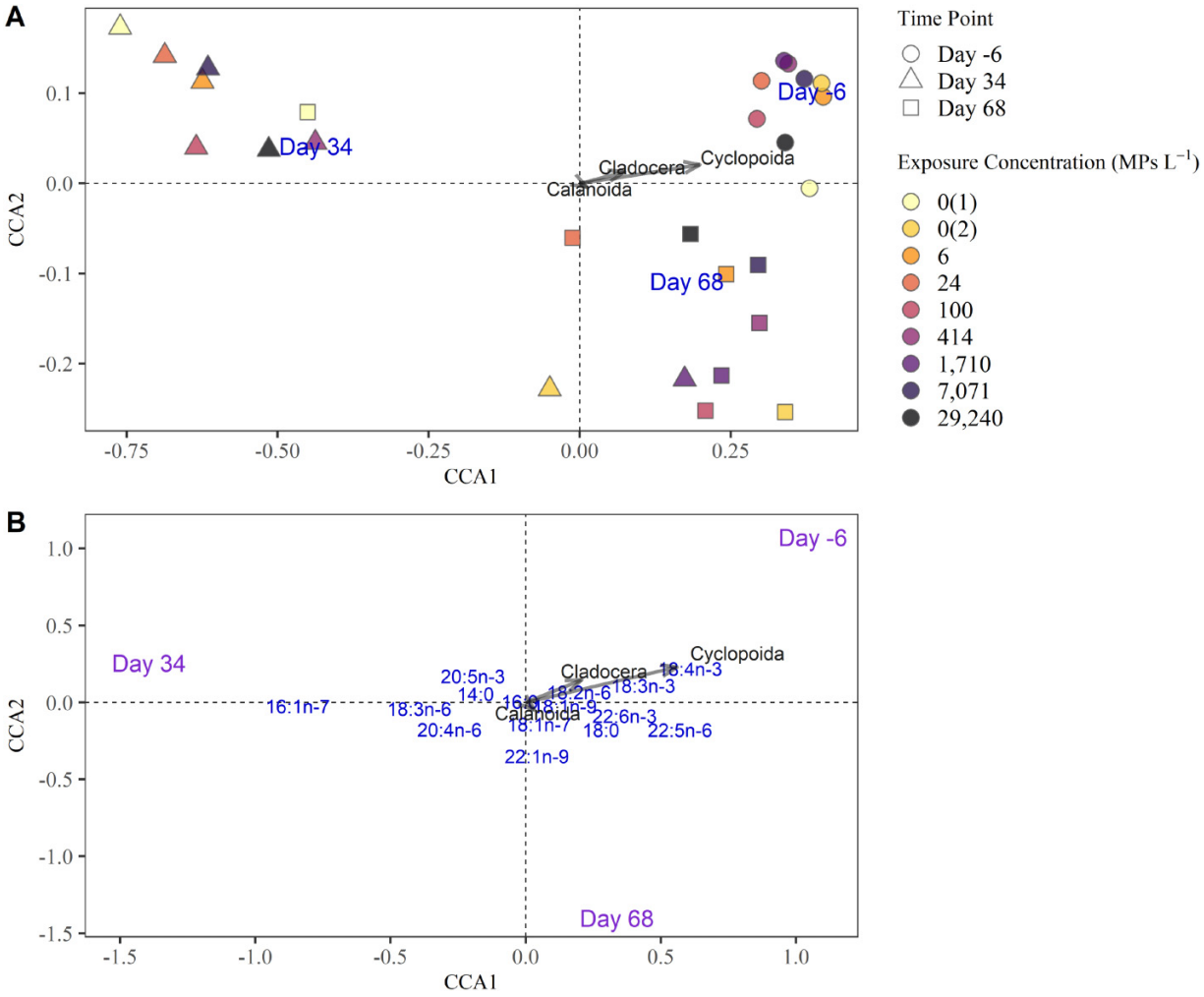
but there was a higher concentration of total fatty acids at day 68 ($z = 2.79$, $p = 0.005$), with a predicted concentration of 71.07 mg g^{-1} dried sample compared with 54.33 mg g^{-1} at day 34. Because of this difference in total fatty acids, we report absolute rather than relative concentrations for the major groupings to better relate to zooplankton biomass. Individual fatty acids by concentration and according to treatment are shown in Fig. S8. Across all treatments, SFAs decreased from day -6 to day 34, then remained similar at day 68, at 31.7 ± 3.8, 17.5 ± 3.5, and 22.5 ± 4.8 mg g^{-1}, respectively (Fig. S9). MUFAs remained consistent over the three timepoints, with concentrations of 14.3 ± 1.6, 15.7 ± 3.1, and 13.1 ± 2.5 mg g^{-1}. Total n-6 PUFAs decreased from day -6 to day 34, then increased slightly by day 68, at 16.4 ± 1.7, 7.1 ± 1.7, and 11.8 ± 4.0 mg g^{-1}. Total n-3 PUFAs also decreased by from day -6 to day 34, then increased again at day 68, with values of 45.3 ± 6.3, 13.1 ± 4.2, and 22.7 ± 6.7 mg g^{-1}. There was no effect of nominal MP concentration ($F_{1,20} = 0.51$, $p = 0.53$), but a strong effect of date ($F_{2,20} = 10.92$, $p = 0.001$) on zooplankton fatty acid composition according to CCA ($R^2 = 0.64$, adjusted $R^2 = 0.53$) and post hoc permutation tests of the marginal effects of terms. There was no significant relationship between fatty acid composition and cladoceran ($F_{1,20} = 0.47$, $p = 0.61$), calanoid ($F_{1,20} = 0.44$, $p = 0.58$), or cyclopoid biomass ($F_{1,20} = 0.32$, $p = 0.72$). Plotting the individual samples scores with treatment and timepoint centroids demonstrated strong separation by timepoint, with low spread when the zooplankton were first added, but high spread at days 34 and 68 (Fig. 4A). Plotting the fatty acid scores alongside the timepoint centroids and the zooplankton biomass vectors primarily showed a positive association between the MUFA 16:1n-7 (palmitoleic acid) at day 34. The plot also suggests a positive association between ALA and 18:4n-3 (stearidonic acid) and day -6, potentially associated with greater cladoceran and cyclopoid biomass and a negative association between ARA and 18:3n-6 (γ-linoleic acid) and day -6 (Fig. 4B).

For the zooplankton at days 34 and 68, there was no effect of MP concentration on proportional DHA ($p = 0.55$), EPA ($p = 0.69$), or ARA (0.20), but there were differences by date, with more DHA at the end and less EPA and ARA ($p < 0.001$ for all; Fig. 5). There was no effect of MP concentration ($p = 0.56$) or date ($p = 0.49$) on absolute concentrations of ARA. There was no effect of MP concentration on absolute concentrations of EPA ($p = 0.54$) but there was a predicted reduction in EPA from 7.17 to 5.74 mg g^{-1} at the day 68 relative to day 34 ($p = 0.02$). There was no effect of MP concentration on DHA ($p = 0.57$) but there was a predicted increase in DHA of 2.67–6.41 mg g^{-1} from the days 34 to 68 ($p < 0.001$).

Discussion

We did not find that environmentally relevant concentrations of MP altered yellow perch diet or the fatty acid composition of yellow perch dorsal muscle or of zooplankton communities during our 10-week mesocosm experiment. Absolute concentrations of HUFAs were also unaltered in either biological matrix, besides yellow perch muscle DHA increasing slightly with MP exposure. We interpret this single response only as preliminary evidence with unclear implications. Similar total fatty acid content in yellow perch muscle across MP treatments suggests that toxicological effects, including food dilution, were minimal. Differences in final yellow perch body weight were primarily explained by a negative correlation with the number of surviving individuals in each mesocosm, probably due to reduced competition in high mortality mesocosms (i.e., density-dependence). These results do not support any effects of MPs on aquatic food web structure at even high levels of MP exposure. Concentrations of MPs in the yellow perch digestive tracts correlated with nominal exposure concentrations and, in the 29 240 particles L^{-1} treatment, were as great as 607 particles $individual^{-1}$ (Rochman et al. 2024), despite an average water column concentration of 246 particles L^{-1} and only 0.06 particles

Fig. 4. Results of canonical correspondence analysis (CCA) for the zooplankton compositional fatty acids data with predictors time point (days -6, 34, and 68), nominal microplastic (MP) concentration, and cladoceran, cyclopoid, and calanoid biomass. For zooplankton biomass, samples from day 1 were used to relate to day -6 fatty acids. Plot A shows individual sample scores (the weighted averages of fatty acid scores), with shape and grey hulls separating sampling points, color indicating treatment/mesocosm, with time point centroids in blue and arrows indicating vectors for zooplankton biomass. Plot B shows fatty acids scores in blue as the weighted averages of sample scores, and mesocosm and sampling point centroids in purple. In plot A, a sample located closer to a time point centroid is more likely to be from that time point. In plot B, a fatty acid score found closer to a time point centroid is likely to be positively associated with that time point.

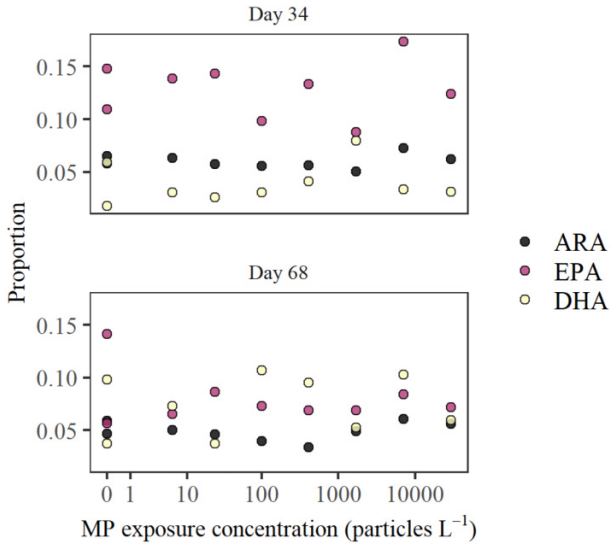


individual^{-1} for zooplankton. This level of contamination in the yellow perch is similar to the highest concentrations reported in fish digestive tracts collected from the environment (Munno et al. 2021) and suggests the yellow perch were feeding to a large extent from the benthos, where MP concentrations would have been highest. However, variable yellow perch survival and body weight in the controls, and high variation among mesocosms may have precluded MP effect detection. Our supplemental feeding of the yellow perch with a high-quality source of fatty acids may have further obscured any nutritional effects which might have propagated through the food web if the fatty acid composition of biofilm or phytoplankton communities (which we did not measure) were affected.

The lack of a response to MP exposure in yellow perch or zooplankton fatty acids is in line with other results from this experiment. Phytoplankton and zooplankton community composition and biomass was not affected by MP treatment, with one exception being a positive relationship between MP concentration and zooplankton abundance on day 33 (Langenfeld 2023) and otherwise reflected changes through time rather than a concentration response. See the supplementary materials (S3) for additional discussion relating to the zooplankton and phytoplankton communities.

Our perch data are in line with other findings that MP exposure does not cause consistent directional responses in body length, mass, or condition in fish, although most experiments have been conducted in laboratory settings (Hossain and Olden 2022). It is possible that 10 weeks was insufficient time to witness significant impacts of MP on growth, as might be predicted by food dilution, although the yellow perch were small enough that they were probably dedicating the major-

Fig. 5. Relative concentrations of arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in the zooplankton samples across mesocosms, according to nominal microplastic (MP) exposure concentration (*x*-axis, log-scale) at days 34 and 68.



ity of their consumed energy to somatic growth (Rennie and Venturelli 2015). Lu et al. (2022) exposed yellow perch in a laboratory study over 9 weeks to HDPE at 0%, 1%, 2%, 4%, and 8% of diet by weight and found that while exposure did not affect survival or growth, fish fed the highest amount of high density polyethylene (HDPE) had lower protein and ash content, increased liver weight and decreased liver lipid concentrations. All exposure levels caused changes in the communities of intestinal microbiota. It is difficult to directly compare exposure in our studies, as Lu et al. did not analyze any fish for gut contents immediately following exposure, but 8% of diet by mass, where they saw most results, is quite high and likely at or beyond the level of exposure in our highest concentration treatment.

There are limited studies on the effects of MPs on fish fatty acid composition in different tissues, although a variety of responses have been demonstrated for other contaminants. These findings include decreases in PUFA and increases in SFA in fish exposed to organic and inorganic pollutants, although EPA and DHA have also been found to increase with tissue levels of persistent organic pollutants (Geng et al. 2015; Filimonova et al. 2016). Our results do not suggest a strong effect of MPs or their additives on yellow perch or zooplankton as fatty acid sources in food webs. However, we only considered the yellow perch fatty acid composition in muscle tissue, while liver tissue might have been more representative of shorter term changes in dietary fatty acids (Mohan et al. 2016). Our results also do not suggest the presence of food dilution, although it is possible that food dilution effects would have emerged if food was more limiting (Piccardo et al. 2020; Lyu et al. 2022).

The increase of yellow perch dorsal muscle DHA with MP exposure warrants future exploration. We propose several explanations but emphasize that they are speculative. As the effect size was low, it remains possible that this was a statistical artifact. If a true effect, it is possible that the yellow perch either increased their retention of DHA in their dorsal muscle tissue, upregulated conversion to DHA from EPA, or had greater access to DHA in their diet. Hanachi et al. (2021) found that rainbow trout (*Oncorhynchus mykiss*) exposed to PS and chlorpyrifos displayed an increase of DHA in muscle tissue and suggested that this represented an oxidative stress response (Hanachi et al. 2021). One of the consistently witnessed effects of MP exposure on fish is an increase in oxidative stress (Kim et al. 2021), and there is evidence DHA can act as an antioxidant (Liu et al. 2014; Rabeh et al. 2021), so it is feasible that yellow perch upregulated retention or synthesis of DHA. Calanoid copepod abundance also did trend higher with MP concentration through the experiment (Langenfeld 2023), and these copepods are higher in DHA than cladocerans or smaller copepods (Persson and Vrede 2006; Hiltunen et al. 2015). Although we did not detect individuals of this group in our limited diet study, fatty acids integrate diet over time, so the yellow perch could have eaten more calanoid copepods in the higher MP treatments earlier in the experiment. Biofouled MPs may have also facilitated increased consumption of algae high in fatty acids, including DHA, increasing yellow perch dorsal muscle DHA in the higher treatment yellow perch, as well as contributing to their ability to maintain fatty acid composition in their tissues where food dilution might otherwise be occurring. There is some evidence of this effect for *Daphnia* (Canniff and Hoang 2018; Amariei et al. 2022), but it has not yet been explored for fish.

This work suggests that fish and zooplankton communities can tolerate MP concentrations well beyond those currently found in the environment. However, there are some important considerations and limitations to our findings. Measured water concentrations of MPs in the highest treatment mesocosm were two orders of magnitude lower than nominal concentrations. Based on size of the yellow perch (Graeb et al. 2006), prey taxa detected in their stomach, including chironomids, as well as the presence of PET particles, it is highly likely that they were feeding along the walls and bottoms of the mesocosms and targeting benthic invertebrates. This would have exposed them to higher concentrations of MPs than what was in the water column. We did not measure fatty acid composition in the epifaunal communities living on the walls and bottom of the mesocosms, so it is unclear how these communities responded to MP exposure. However, in the highest treatment, MP concentrations (mostly PE) in the wall-attached biofilm increased over time and reached concentrations of 282 907 particles m^{-2} at 0.2 m and 1205 310 particles m^{-2} at 1.2 m depth by week 9 of the experiment (Rochman et al. 2024), supporting the idea of high MP exposure for the yellow perch feeding on the walls and bottom. Pelagic zooplankton would have experienced lower exposure amounts than yellow perch, which could explain a lack of effects on their communities and low ingested MP levels. Our results for many variables, including fatty acids and yellow perch body weight, were also highly variable and results from the two control mesocosms included the lowest and highest

values. We suspect density-dependent growth occured in the mesocosms, driven by early experimental differences in yellow perch mortality due to handling stress, which may have masked any MP exposure response.

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Data availability

All data and code used in this study are available on Borealis: https://doi.org/10.5683/SP3/AUK12U.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2024-0149.

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