Gorule, P.A., Šmejkal, M., Tapkir, S., Stepanyshyna, Y., Stejskal, V., Follesa, M.C., Cau, A., 2024. Long-term sublethal exposure to polyethylene and tire wear particles: Effects on risk-taking behaviour in invasive and native fish. Science of the Total Environnment 908, 168233.

The publisher's version is available at:

doi:10.1016/j.scitotenv.2023.168233

Long-term sublethal exposure to polyethylene and tire wear particles: Effects on risk-taking behaviour in invasive and native fish

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Abstract

Anthropogenic polymer particulates pollute even the most remote ecosystems and may compromise the behaviour and movement skills of organisms. It is expected that invasive species cope better with pollution than native species (i.e., pollution resistance hypothesis). In this study, invasive gibel carp (*Carassius gibelio*) and native crucian carp (*Carassius carassius*) were used as model organisms. Specimens were fed daily with food pellets (1% body weight) added with 0.1% polyethylene (PE), TWPs and control. Their behavioural parameters were compared before and after 14 and 60 days of exposure. Additionally, we evaluated burst swimming capacity after 60 days of exposure to the treatments. The fishes exposed to the PE and TWPs treatments showed significant trends toward increased boldness scores and, in the PE treatment, higher utilization of the open field, and both behavioural changes are associated with higher risk-taking. Invasive gibel carp had substantially better swimming performance than crucian carp, but the expected trend in relation to the treatments was not found. Fish exposed to sublethal doses of PE and TWPs showed signs of behavioural changes after two months of exposure that may affect risk-taking behaviour, which might impact species interactions with predators.

Keywords

habitat degradation; pollution; invasive species; behavioural changes; invasion ecology

1. Introduction

Plastic production worldwide has resulted in the generation of over 8 billion tons of waste; over 80% of it is currently dispersed across all environmental matrices (Geyer et al., 2017). Plastic waste ends up in water bodies such as lakes and oceans, with rivers playing a major role in its transport and transfer across different habitats (Cau et al., 2022; McNeish et al., 2018; Palmas et al., 2022). The scientific community has paid particular attention to plastics that deteriorate and fragment into smaller particles, generating microplastics (MPs): plastic particles with dimensions comprised between 1 μm and 5 mm (Frias and Nash, 2019). Generally, MPs in rivers originate from anthropogenic land-based sources due to industrial activities and population densities (Birch et al., 2020). Microplastics can be found in soil, atmosphere, marine and freshwater sediments, surface or ground water and biota (Boyle and Örmeci, 2020; Hidalgo-Ruz et al., 2012; Lee et al., 2013; Liu et al., 2021; Robin et al., 2020). Microplastics are ingested by plethora of aquatic organisms and may cause mechanical damage to tissues but also physiological or behavioural responses such as immune system disturbances, oxidative stress, developmental defects, growth suppression and abnormal feeding selectivity (Au et al., 2015; Caccamo et al., 2016; Cau et al., 2023, 2020; de Sá et al., 2015; Espinosa et al., 2019; Rist et al., 2016; Zhang et al., 2022).

Among conventional plastics that dominate the world market, polyethylene (PE) has a major role. PE has experienced widespread use since the 1950s in packaging products with usually short shelf lives, such as plastic bags, bottles, cups and containers. Thus, it is not surprising that it is one of the most recurrent types of MPs retrieved in aquatic environments, regardless of the matrix of dominium explored (Kumar et al., 2021).

Tire wear particles (TWPs) are generated from the mechanical abrasion of tire material during use on roads and have gained considerable attention as part of MPs that contaminate aquatic environments (Wagner et al., 2018). Tire wear particles are dispersed in aquatic environments through various pathways, such as wastewater, road water runoff, and atmospheric deposition (Kukutschová et al., 2011; Sugiura et al., 2021; Ziajahromi et al., 2020). It is estimated that approximately 500,000 tonnes of TWPs are generated annually in the European Union (EU) alone, and 50–140,000 tonnes are released annually in EU surface waters (Hann et al., 2018; Page et al., 2022). TWPs are made of a complex mixture of rubber, e.g., styrene butadiene, embedded asphalt, pavement minerals and other minerals (copper and zinc) (Eisentraut et al., 2018; Panko et al., 2013). Till date, specific chemical components of TWP have been the main focus for ecotoxicity studies, while studies that incorporate the physical components of the contaminant are quite rare and should be prioritized (Wagner et al., 2018). TWPs can also include metals (e.g., copper) that can affect both biological (i.e., reduced growth) and ecological (i.e., prey–predator interactions, behavioural patterns, swimming performance) traits (Gosavi et al., 2020; Siddiqui et al., 2022).

While great effort has been made over the last years to document the contamination by MPs in aquatic environments, knowledge on the effects that exposure to specific polymers might have on aquatic organisms is still scarce (Bucci et al., 2020), even in freshwater environments, where PE and TWPs are more prone to be collected and transferred (Cunningham et al., 2022; Wagner et al., 2018). Behavioural changes in response to exposure to pollutants are becoming an increasingly important topic in ecotoxicology (Oulton et al., 2014). As a general pattern, behavioural changes may occur even with small quantities of pollutants (Bae and Park, 2014), as already documented in invertebrates (Van Colen et al., 2020), amphibians (Araújo and Malafaia, 2020) and fish (Critchell and Hoogenboom, 2018). Despite the fact that changes in animal health may not be noticeable, behavioural changes may have severe consequences on animal interactions within the community (Scott and Sloman, 2004), thus allowing us to identify and infer sub-individual effects that eventually can alter population or even community dynamics due to their adaptive and maladaptive responses to the pollution in the wild and their resistance to the pollutant due to multiple stressors along the biological hierarchy (Ashauer and Jager, 2018; Sih et al., 2011).

As a potential cumulative effect of MP contamination in aquatic environments, the "pollution resistance hypothesis" postulates that invasive species may have a competitive advantage in environments that are polluted or contaminated with toxic substances (Crooks et al., 2011). The hypothesis proposes that invasive species have evolved traits that enable them to tolerate or detoxify pollutants, giving them a competitive edge over native species that may be more susceptible to pollution (Crooks et al., 2011; El Haj et al., 2019; Varó et al., 2015). This may be due to the nature of invasive species establishment, where many more species are translocated and only a handful become successful and invasive (Blackburn et al., 2011; Sakai et al., 2001). As invasions often start near urban areas with high levels of pollution and environmental change (Qiao et al., 2022), this can act as a selective mechanism for pollution-resistant invasive species (Camacho-Cervantes and Wong, 2023). Hence, to address this hypothesis with declining native species and corresponding invasive species, we used the native crucian carp (*Carassius carassius* L., 1758), a sharply declining species in European waters due to invasion by gibel carp (*Carassius gibelio* K., 1782) (Jeffries et al., 2016; Kottelat and Freyhof, 2007). The invasive gibel carp was imported into Romania in the mid-20th century (Szalay, 1954; Tóth, 1976) and has since spread across the European continent (Kottelat and Freyhof, 2007; Perdikaris et al., 2012; Ribeiro et al., 2015; Wouters et al., 2012). During gibel carp invasion, most crucian carp populations were extirpated due to the higher competitive ability of the invasive carp (Tapkir et al., 2022).

With these premises, the present study compares the behavioural and performance responses of two *Carassius* species exposed to 0.1% mass of PE and TWPs. We conducted manipulative experiments to test as to whether exposure could i) alter fish behaviour either towards more cautious or bolder attitude; ii) reduce fish swimming capacity, thus potentially affecting predator–prey relationships and iii) differ in these effects among the native and the invasive species.

2. Materials and Methods

2.1. Preparation of microplastics

To realistically simulate environmental scenarios, manipulative experiments described in the present work were conducted using particles produced from discarded commercial tires and plastic bottles. For both polymers, toxicity levels are known to vary across different stages of weathering (Arp et al., 2021; Halle et al., 2021); thus, in the present study we used end-life tires, that do show a different toxicity compared to new tires, and bottles retrieved from the environment. Both items were retrieved from aquatic environments, during river clean-up for macrolitter. This was done to ensure that tires and plastics underwent not only typical usage but also environmental weathering (i.e., photo-, thermo- and mechanical deterioration). In detail, PE-based MPs were produced from a single PE item collected during the abovementioned activities and analytically characterized via micro Fourier transform infrared spectroscopy (μFT-IR) (spectra available in Supplementary Figure S1). Scanning electron microscopy (SEM) was used to produce detailed images and evaluate morphological features of TWPs (Supplementary Figure.S2), for their shape to be as similar as possible similar to those produced by the friction of tires on asphalt Knight et al.(2020);. Kreider et al.(2010)The elemental analysis of the particles was carried out by X-ray Energy Dispersive Spectrometer of SEM (Supplementary Figure S3). The elemental composition TWPs observed on the surface of the particles (Supplementary table T2) are used in tire manufacture Yang et al.(2022). The organic constituents of the TWPs were analysed with a micro-furnace pyrolyzer frontier coupled to gas chromatography combustion isotope ratio mass spectrometry (GC/IRMS), the selected markers used and the compounds detected are provided in (Supplementary Table T1).

Effects on biota can be caused by physical interactions between particles and organisms, according to particle size and shape, and by associated compounds released from the particles (Skjolding et al., 2016). Thus, it is crucial to create experimental designs that allow scientists to properly discriminate either effects caused by physical factors (size, volume and shape of particles) or from chemical factors such (e.g., different polymers, production additives, etc.), within the same level of the biological hierarchy (Bucci et al., 2020). To meet this need, particles of both polymers were manually grated to create irregularly shaped particles (especially for TWPs, that derive from tires abrasion with asphalt) and then sieved to an expected size range comprised between 70 and 210 μm (estimated relative abundance within this size range is reported in Supplementary Table X). This size range was considered relevant for the purpose of the experiment since it simultaneously encompasses the most common shape and size range of runoff and/or shredded tires (Charters et al., 2015).

2.2. Diet

The feeds were manufactured in the laboratory at the Institute of Aquaculture and Protection of Waters, University of South Bohemia. A hammer mill grinder (Mistral 50 L, River System SRL, Italy) with an 18-mm mesh was used to grind commercial feed (C-3 Carpe F, Skretting, Stavanger, Norway). A portion of the ground feed (1000 g) and PE (1 g) or TWPs (1 g) were thoroughly mixed in a container to achieve homogeneity and the experimental groups 0.1% TWPs and 0.1% PE. Subsequently, 0.2 L of warm water (40 °C) was gradually added and thoroughly mixed into the feed. Water was added until the mixture become adhesive. Then, the mixture was passed through the mixer, mini pelleting machine (2-mm diameter plate, Bottene, Bottene Fratelli Snc, Italy) and cutter (Cutter LT3, FAC srl, Italy) to form pellets. The pellets were then placed in paper-lined trays and dried in an air oven for 24 h at 55 °C. The oven was shut off after the feeds were dried and they were left to cool to room temperature (20 °C). Feeds were stored at -20 °C in plastic bags until fed to the fish. The control diet contained 0% plastic, and it was prepared using the same procedure to achieve similar surface textures and to avoid any unforeseen interferences.

2.3. Fish collection and maintenance

Live crucian carps (n=60) with an average size of 93.5 ± 6.5 mm (range 81–108 mm) were collected from Pavlov u Herálce (49.5039411N, 15.4285000E), and live invasive gibel carp (n=60) with an average size of 91 ± 5.7 mm (range 81–105 mm) were collected from the same site. To prevent mortality, the fish were transported to the laboratory in plastic barrels filled with dechlorinated oxygenated water. Fish were kept in glass aquariums (64 x 60 x 45 cm) filled with 130 L of clean, oxygenated 20 °C water in the laboratory in groups of ten fish. The photoperiod was 12 h light (9:00 a.m. light on) and 12 h dark (9:00 p.m. light off). The aquariums were fitted with aerators for continuous oxygenation, and water from all the aquariums was changed every 14 days. Fish were allowed to acclimatize for 30 days prior to the experiment.

*2.4. Experimental design*A total of 120 fish; 60 *(Carassius Carassius)*, 60 (*Carassius gibelio)* with similar body weights were distributed into 12 experimental tanks (n=10 fish per tank). The native and invasive species were kept separately in three treatments: i) control treatment, ii) PE treatment, and iii) TWPs treatment, using 20 fish per treatment for each species (Supplementary Fig S.4). The fish were anaesthetised with MS-222 before tagging, and their standard length (in mm) and weight (in g) were recorded. During tagging, 3-4 scales were removed, and a 2-3 mm vertical incision was made 3 cm posterior to the pelvic fin (Šmejkal et al., 2019). Then, a passive integrated transponder tag (PIT tag, Oregon RFID, Oregon, USA; half-duplex; length: 12 mm; diameter: 2.12 mm; weight: 0.1 g; ISO 11784/11785 compatible) was inserted into the body cavity. The fish were given a healing time of 30 days before starting the experiment. The fish were exposed for 14 and 60 days to the three different treatment diets (control, PE, and TWPs) and were fed daily at 1% of body weight supplied once a day. Fish were monitored throughout the experiment for any potential indications of poor health status (i.e., feeding behaviour, swimming activity and condition of fins). Physical and chemical water parameters (pH and dissolved oxygen mg L^{-1}) were measured on a weekly basis using a water quality sensor (Digisens-OPTOD, Ponsel, France) and a computer running CalSens 1.4 software (Aqualabo, France).

2.5. Fish behaviour assays

2.5.1 Boldness – emergence time

The scoring behaviour was performed in a rectangular arena (57 cm long × 57 cm wide) filled with dechlorinated tap water. A start box was placed at one end of the arena, and an air stone was placed for aeration. The start box had a sliding door on one side operated by a pulley (Supplementary Figure S5). The body weight, standard length and total length of each fish were measured one day prior to the experiment. The fish were fed 24 h before the experiment. The test fish were removed from the fish tanks using a hand net and placed into a start box. Using a pulley, the adjustable doors were maintained closed to keep the fish out of the test room. The fish were allowed to acclimatize for 10 min, and later, with the help of a pulley, the arena door was gently opened, allowing the fish to emerge in the test arena. The videos were recorded for 20 min after opening the arena door. To minimize the effects of human observers on fish behaviour, a webcam (Logitech

C270, Laussane, Switzerland) was positioned directly above the apparatus and utilized to record videos. Videos were recorded for fish emergence into the test arena, and the emergence time was noted when the complete fish (i.e., fish fully visible in the arena) exited the start box (Tosetto et al., 2017). The emergence time and boldness have an inverse relationship, i.e., a decrease in the emergence time leads to an increase in the boldness value. The highest scores, i.e., 1200 seconds, were given to the fish that did not appear in the test arena.

2.5.2. Open field test

The open field was represented by a rectangular arena (57 cm long × 57 cm wide) and was filled with oxygenated water. The bottom of the apparatus was divided into the centre, which made up 60% of the whole arena, and the periphery, which made up 40% (Supplementary Figure S6) (Araújo and Malafaia, 2020). Body measurements (weight, standard length and total length) of each fish were measured one day prior to the experiment. Fish were fed 24 h before the experiment. The fish were individually introduced in the centre of the arena and allowed to acclimatize for 10 min and later recorded for 20 min using a webcam (Logitech C270, Laussane, Switzerland) connected to a computer running iSPY software (www.ispyconnect.com). The distance travelled by the fish (measured in cm) was calculated as well as the ratio of locomotion in the arena centre to total locomotion during the test period. Video recordings were analysed using LoliTrack 5 (Loligo Systems, Tjele, Denmark).

2.5.3. Swimming performance

Evaluation of swimming performance and oxygen consumption was performed in a 5-L (testing section 28 x 7.5 x 7.5 cm) Steffensen-type swimming tunnel respirometer (Loligo systems, Tjele, Denmark) submerged in a buffer tank that was connected to an aerated temperature-controlled 50-L reservoir tank allowing continuous water exchange (Supplementary Figure S7). The swimming chamber was connected to a buffer tank through a flush pump (5 L/min, Eheim GmbH, Deizisau, Germany) to ensure an adequate dissolved oxygen concentration for swimming performance testing. Flow calibrations were performed using a handheld flowmeter (Flowtherm, Höntzsch GmbH, Waiblingen, Germany). The level of dissolved oxygen in the test chamber was kept above 70% throughout the experiment (Tran et al., 2021). The temperature and dissolved oxygen in the swimming chamber were continuously monitored using a fibreoptic oxygen probe and a temperature probe coupled to a Witrox 1 sensor meter (Loligo Systems, Tjele, Denmark). The water temperature was maintained at 20 ± 0.1 °C. The tank was covered with paper to prevent disturbance from the outside. The water flow and dissolved oxygen in the swimming chamber were monitored and controlled by the system via AutoResp software (Loligo Systems, Tjele, Denmark).

2.6. Experimental protocol

A total of 120 fish (20 fish/diet group) were used in swimming tests following 24 h without feeding. The standard length (in mm) and weight (in g) were recorded prior to the experiments. Fish were randomly and rotationally selected from the species and treatment groups (a single fish was measured at a time) and identified by the PIT tag number. Individual fish were transferred to the swimming tunnel and allowed to acclimatize to the test conditions for 10 min with a water flow velocity of 10 cm s^{-1}. The tunnel was closed to avoid water exchange with the surrounding tank. The initial velocity was set at 10 cm s^{-1} and increased by 5 cm s^{-1} increments every two min, terminating at a maximum of 150 cm s^{-1} or when the fish was exhausted (stopped swimming). The small increments of velocity and time in our protocol were set to minimize stress on the tested fish, and the protocol follows Tran et al. (2021). The swimming test was terminated when the test fish remained at the rear grid for more than 10 s without any activity. The critical swimming speed (U_{crit}, cm s^{-1}) was calculated using the previously described equation (Brett, 1964):

$$U_{crit} = U_{max} + (T_{max}/T_{interval} * U_{interval})$$

where U_{max} is the highest velocity recorded at fatigue (cm s^{-1}); $U_{interval}$ is the velocity interval (5 cm s^{-1}); T_{max} is the time spent at fatigued velocity; and $T_{interval}$ is the time interval (2 min).

2.7. Data analysis

Possible effects of species, treatment, initial standard length (SL) and time of exposure on the behavioural parameters (emergence time, swimming activity and open field utilization) were analysed using linear mixed effects models (LMMs). Individual fish identity was used as a random intercept in the models to account for the among-individual differences in behaviour. Model selection based on the corrected Akaike information criterion (AICc) was used to identify the most parsimonious model with the lowest AICc and other plausible models (ΔAICc ≤ 2) for the data (Burnham and Anderson, 2002). Four models per behavioural variable were fit, including the full model with species, treatment, initial SL and the statistical interaction between treatment and time, and possible submodels (behaviour parameter ~ species; species + treatment; species + treatment + SL) were among the candidate models of individual fish behaviour in the experiment. The analyses were conducted in R (R Core Team, 2022) using the packages nlme and jtools (Long, 2022; Pinheiro et al., 2021). To gain better insight into changing behaviour across different treatments and times of exposure, forest plots were obtained using log-transformed ratios of mean response variables (i.e., boldness, open field utilization and swimming activity) in fish fed contaminated pellets (PE and TWPs). Forest plots were generated using R Studio (R Core Team 2016) through the meta-analysis packages "metafor" (Quintana, 2015; Viechtbauer, 2010) and "robumeta"; the script was created by Quintana (2015) and modified using the log-transformed ratios of means as the effect size.

To test whether exposure to TWPs or PE reduced the fish swimming capacity using cm s^{-1} and body lengths s^{-1}, a general linear model (GLM) was constructed with swimming performance as the response variable and species, treatment and fish weight as explanatory variables.

3. Results and discussion

No fish mortality under experimental conditions was observed. Comparison of LMMs of fish emergence time showed that the most parsimonious model was the most complex and included the explanatory variables species, initial SL, treatment, interaction of treatment and time and fish ID as a random intercept, with an R^2 of 0.27. The effect of species and SL was not significant in the model, nor was the effect of treatment, but the emergence time significantly decreased as a result of the interaction between treatment and time at 60 days, being lowest in both the PE and TWPs treatments (Table 1, Figure 1). Fish behaviour in the open field test was best explained by a simple model including only species and treatment (AIC=413); however, fish behaviour in the open field test was best explained by the most complex model that also involved SL and time effects, with an R^2 of 0.36 (AIC=420). Species identity was significant in the model, and a further significant change was observed as a result of the interaction between time at 60 days and PE, with higher utilization of the open field in crucian carp than in the other groups and experiment time (Table 2, Figure 2). In the case of fish activity, the most parsimonious model was a simple model with only the explanatory variable species; however, the AIC was very similar for all models. The most complex model showed a significant effect of time at 14 and 60 for TWPs (Table 3, Figure 3).

The most complex GLM with fish swimming speed was chosen based on the AIC, and it showed high significance of species on maximum swimming speed ($t = 9.34$, $p < 0.001$; Figure 4) and a positive effect of the TWPs treatment on swimming speed in gibel carp ($t = 2.22$, $p = 0.03$; Figure 4). The best model with the response variable swimming speed expressed as body lengths s^{-1} was a GLM with species as the explanatory variable ($t = 9.63$, $p < 0.001$).

Forest plot analysis showed that, compared to controls, PE contamination led to a significantly negative effect (i.e., decrease in exploratory behaviour) on crucian carp in the open field test, after 14 days of exposure (LnR = -0.49 ± 0.28; 95% CI) and decrease in the emergence time of the crucian carp after 60 days (LnR = -0.58 ± 0.28; 95% CI). In contrast, PE led to a positive (i.e., decrease in the emergence time) effect on bolder behaviour for gibel carp after 60 days (LnR = 0.43 ± 0.46; 95% CI; Figure 5). TWPs exerted a significantly positive effect on gibel carp in the open field test after 14 days of exposure (LnR = 0.20 ± 0.19; 95% CI), and a significant negative effect on emergence time after 60 days of exposure (LnR = -0.47 ± 0.46; 95% CI; Figure 5).

Our results suggest that within laboratory settings, sublethal doses of PE and TWPs may cause fish to change their behaviour in the direction of more active and bold behaviour, especially in native crucian carp. In the present study, we also obtained highly significant results in the case of species for maximum swimming speed, indicating a positive shift caused by TWPs exposure. The swimming speed of the invasive species increased significantly, which indicates that there is a possible threat to species interactions, since locomotion is being affected by the experimental treatments, especially TWPs. Such induced behavioural changes may lead to more risk-taking behaviour in the wild, with potential repercussions for predator-induced mortality rates (Houston et al., 1993; Hulthén et al., 2017; McCormick et al., 2018). The ecotoxic effect of PE MPs on different fish species was addressed in a laboratory study, and trophic transfer of PE from *Poecilia reticulata* (primary consumer) to *Danio rerio* adults (secondary consumer) resulted in behavioural changes such as deficits in the anti-predatory defensive response in the organisms in the upper trophic level (da Costa Araújo et al., 2020). The study of Araújo and Malafaia (2020) suggested that PE MP accumulation in the tadpole *Physalaemus cuvieri* affects the locomotion ability, anxiogenic behaviour, and antipredator response deficit in anurans exposed to potential predators.

3.1 Evidence of the pollution resistance hypothesis

Our results also provided support for the pollution resistance hypothesis, showing that invasive species reacted to the toxicity of PE and TWPs with less pronounced changes in behaviour triggered by PE and TWPs compared to native species. Bold individuals of zebrafish were found to have a higher exposure burden to MPs than shy individuals, which is contrary to the common perception of better survival chances among bolder individuals (Chen et al., 2022). The pollution resistance hypothesis is likely valid only in selected scenarios. The opposite example is that native *Artemia* species are extremely resistant to Hg, which prevents the invasion of non-native *Artemia franciscana* (Pais-Costa et al., 2020).

Most of the available knowledge highlights the low sensitivity of gibel carp to environmental contaminants and pollution (Gkelis et al., 2006; Kagalou et al., 2008; Perdikaris et al., 2012), while comparative information on the two study species in relation to pollutants is still missing. The results presented here further corroborate the higher resistance of invasive species to MPs and provide insights into the potential effects that, synergically with other disturbances, could strengthen biological invasion and replacement in European freshwater ecosystems. While the replacement of native fish by invasive species likely occurs for many reasons, including the competitive abilities of the two species (Tapkir et al., 2022), the most pronounced decline in crucian carp was observed in degraded and suboptimal habitats, such as the oxbows of large river systems (Buj et al., 2023; Kottelat and Freyhof, 2007; Lusk et al., 2010), which supports the pollution resistance hypothesis.

3.3 Toxicity of PE and TWPs: current knowledge

The compounds leached from tires into water include a multitude of chemicals (Chibwe et al., 2022; Halle et al., 2021), which have potential toxic effects on fish behaviour and performance (Chang et al., 2023; Chibwe et al., 2022). Toxicity studies documented differential effects of leachate originating from worn and new tires, with the latter showing long-term acute toxic effects in freshwater crustacean *Hyalella azteca* (Halle et al., 2021). The toxicity of tire leachate has also been addressed in early life stages of zebrafish (*Danio rerio*) embryos, which displayed a dose-dependent reduced swimming performance (i.e., velocity, locomotive behaviour, total distance travelled) (Varshney et al., 2022). Studies also indicated the toxic effect of TWP leachate on marine phytoplankton, the base of marine food webs, with a reduced growth rate observed in three phytoplankton species, namely, the cryptophyte *Rhodomonas salina*, the diatom *Thalassiosira weissflogii* and the dinoflagellate *Heterocapsa steinii* (Page et al., 2022).

In adult zebrafish, the exposure to 10–600 μm PE particles at a concentration of 2 mg L^{-1} resulted in abnormal behaviours such as erratic movement, seizures and downwards tail bending (Mak et al., 2019). The short-term trophic transfer of MPs from beach hoppers to fish did not affect fish behaviour; however, there was a shift in boldness with fish becoming shyer due to changes in diet (Tosetto et al., 2017).

The research conducted thus far indicates that the effect of TWPs, PE and other pollutants on behaviour may vary depending on the fish species tested. These studies are of ecological importance; longer exposure to pollutants can disturb the fitness, cognitive, physiological and behavioural patterns of fish from an early stage of life (Jacquin et al., 2020). Accumulation of these pollutants can result in interpopulation divergence and affect ecosystems disproportionately by selectively removing sensitive species.

3.4 Role of behaviour in predator–prey interactions

In the comparison of invasive gibel and native crucian carp, the study showed that although gibel carp had an overall trend toward higher scores in activity, maximum swimming performance and open field utilization (i.e., more active and exploratory behaviour) in the experiments, gibel carp also took much longer to emerge from the box, suggesting more timid behaviour. During the experiment, there was a likelihood for habituation in fish, e.g., reduced time of emergence and increased activity, which could also diminish the perception of the impacts of PE and TWPs on behaviour. To avoid habituation effects, more natural conditions, such as outdoor mesocosms and telemetry testing, can be used to address behavioural changes (Lennox et al., 2021; Šmejkal et al., 2022). In particular, crucian carp were found to be susceptible to habituation, and their behaviour also changed in the expected direction in the control treatments, with crucian carp showing an overall trend of being substantially more relaxed in the laboratory than gibel carp.

Native crucian carps are usually not strong in avoiding predation (Holopainen et al., 1997), and their main adaptation involves inducible morphological changes in body depth (Brönmark and Miner, 1992; Domenici et al., 2008; Hulthén et al., 2014) or an eventual switch from nocturnal to aperiodic activity (Pettersson et al., 2001). In comparison with crucian carp, invasive gibel carp thrive even in predator-rich environments, such as the main channel and oxbows of the Elbe River, Czech Republic (Daněk et al., 2012; Lusk et al., 2010). The observed change in the behavioural trends in relation to predation risks in crucian carp needs to be tested before drawing any conclusions, but it may have the potential to further weaken its anti-predation skill.

3.5 Relevance for species and environment

Despite the extreme resistance of crucian carp to abiotic factors (Blažka, 1958), native crucian carp is facing a decline due to the introduction and competitive abilities of invasive gibels (Jeffries et al., 2016; Kottelat and Freyhof, 2007; Tapkir et al., 2022). Both native crucian carp and invasive gibel carp are well adapted to the non-favourable conditions of ageing pools of floodplains; e.g., they are extremely hypoxia- and heat-tolerant species (Antonova, 2010; Bundgaard et al., 2020; Jackson, 2000; Karvonen et al., 2005; Piironen and Holopainen, 1986). Furthermore, gibel carp were found to survive ammonia concentrations (12.5 mg L^{-1}; pH 8.6) (Nathanailides et al., 2003) and toxic cyanobacterial blooms (Perdikaris et al., 2012) by storing toxins in the liver and other tissues (i.e., ovaries, brain, intestine, muscle and kidneys) (Gkelis et al., 2006; Kagalou et al., 2008). Such high environmental tolerance to abiotic environmental factors is hypothesized to make gibel carp strong enough to outcompete native crucian carp in its suboptimal and degraded habitat (Kottelat and Freyhof, 2007), rendering gibel carp one of Europe's most effective invaders (Perdikaris et al., 2012). While crucian carp is known to prefer floodplain channels with rooted floating aquatic vegetation (Sayer et al., 2011; Tonn et al., 1992; Wheeler, 2000), gibel carp can withstand man-made as well as natural waterways such as streams, rivers, canals, dams, reservoirs, estuaries and ponds (Tarkan et al., 2012; Vetemaa et al., 2005), which could be exposed to high levels of contamination, and where crucian carp populations dropped in recent decades (Lusk et al., 2010; Lusková et al., 2010).

In the present work, we used a concentration of 0.1% of MPs, incorporated in food pellets. We acknowledge that comparison with environmental concentration of MPs across different matrixes is not feasible, since it is impossible to determine the actual exposure to biota starting from the environmental concentration. However, the very few reported weight-based concentrations of MPs, regardless of the matrix, were higher or similar to those used in the present study, and we thus believe that our exposure concentrations can be considered relevant and useful, particularly for foundational studies such as the present one. In order to more accurately replicate the experimental conditions such as concentration and modes of ingestion in the wild, a more natural scenario and typical ingestion of filter feeders like *Daphnia* can be developed for future work based on these behavioural experiments.With respect to the studied species, it may be expected that native crucian carp receives a slightly higher dose of contaminants than gibel carp since the trophic position of crucian carp is higher (feeding solely on zooplankton and benthic invertebrate prey) (Batel et al., 2016; Farrell and Nelson, 2013; Setälä et al., 2014), while part of the gibel carp diet consists of plant material (de Meo et al., 2022; Özdilek and Jones, 2014; Tapkir et al., 2023).

4. Conclusions and future directions

Available scientific literature provides evidence of acute poisoning from road run-off in the case of TWPs and negative effects associated with the accidental ingestion of PE particles. Our study, on the contrary, documents the long-term effects of sublethal concentrations of PE and TWPs, comparing native and invasive species of carps, within freshwater ecosystems. We observed a tendency of crucian carp to reduce its scores related to cautious behaviour (reduced emergence time and increased use of open space) in the PE treatment. It remains to be tested whether higher doses or longer exposure to pollutants would lead to more pronounced changes and whether such changes would be relevant for interactions with other organisms, especially with predators. Our results, based on relevant sublethal concentrations of TWPs, emphasize the need to further identify the potential effects of the polymers included in MPs.

Plastic production, use and management will likely undergo a deep remodulation that will hopefully result in reduced plastic input into the environment (e.g., global plastic treaty; Bergmann et al., 2022). Ideally, this should apply also to TWPs, which should experience the same remodulation forecasted for plastic. Unfortunately, this cannot be fully true for TWPs because, at present, there are no other options that could replace tires for large-scale road transportation, even though a shift in materials used for their production can be foreseen. However, focusing on the near future, there will likely be a consistent input of TWPs through its numerous pathways that span from atmospheric deposition to runoff waters (Wagner et al., 2018). Therefore, the important goal is to evaluate potential negative impacts on the environment and important changes in species interactions that, in specific cases, can eventually foster biological invasions in some ecosystems.

Acknowledgements

We thank the anonymous reviewers and editorial board member for their constructive comments, which significantly enhanced the quality of the manuscript. The authors are thankful to Zuzana Sajdlová for help with the graphical abstract. We also thank Sakshi Tripathi and Hung Quang Tran for their help in laboratory activities the study. We would like to thank Travis B. Meador for his support in providing the chemical characterization of TWPs through Pyrolysis GC-IRMS. We are grateful to the professional editing service (www.aje.com).

Ethical statement

Funding

The authors thank the Research Programme Strategy AV21 Water for Life for valuable support. PAG is supported by a Ph.D. student scholarship awarded by the Department of Life, Environmental and Drug Sciences of University of Cagliari, funded through MIUR - Italian Ministry for University and Research.

Conflict of interest

The authors declare no competing financial interests.

Ethical approval

The field sampling and experimental protocols used in this study were performed in accordance with the guidelines and permission from the Experimental Animal Welfare Commission under the Ministry of Environment of the Czech Republic (ref. no. CZ 01679). The methods and ethics of the study were approved by the Experimental Animal Welfare Commission of Biology Centre of the Czech Academy of Sciences, and a certificate of approval is available upon request.

Informed consent

Not applicable.

Authors' contributions

Pankaj A. Gorule: data curation; investigation; writing—original draft; visualization; formal analysis; methodology; writing—review and editing

Marek Šmejkal: conceptualization; methodology; data curation; investigation; validation; formal analysis; supervision; visualization; funding acquisition; writing—original draft; writing—review and editing; resources

Sandip Tapkir: formal analysis, writing review and editing

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Vlastimil Stejskal: methodology; data curation; conceptualization; writing original draft; writing—review and editing

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Tables

Table 1. Linear mixed effects model for the boldness-emergence time of gibel carp and crucian carp from the observations recorded for a group of 120 fishes, with 40 fishes each in the control and 14-day and 60-day exposure periods. The estimated parametric coefficients and their significance (coefficients for Species, SL, Treatment and the interaction between Treatment and Exposure Time) in the model are shown. The adjusted R^2 of the model with the random intercept fish ID and dependent variable emergence time was 0.27, and the deviance explained by the ICC values was up to 21%. Significant p values for the explanatory variables were calculated using Satterthwaite d.f.

	Est	S.E.	t val.	d.f.	p
Intercept	18.96	203.77	0.09	174.77	0.93
Species (gibel carp)	47.69	28.24	1.69	116.88	0.09
SL	1.90	2.15	0.88	171.48	0.38
Treatment PE	-23.12	49.36	-0.47	319.08	0.64
Treatment TWPs	18.30	49.36	0.37	319.07	0.71
Treatment control Time 14	-33.41	43.84	-0.76	234.08	0.45
Treatment PE Time 14	-35.23	43.91	0.80	235.26	0.42
Treatment TWPs Time 14	-73.85	43.92	-1.68	235.37	0.09
Treatment control Time 60	-133.43	44.21	-3.02	240.41	0.00
Treatment PE Time 60	-106.37	44.39	-2.40	243.44	0.02
Treatment TWPs Time 60	-182.14	44.23	-4.12	240.78	0.00

Table 2. Linear mixed effects model for the open field utilization of gibel carp and crucian carp from the observations recorded for a group of 120 fishes, with 40 fishes each in the control and 14-day and 60-day exposure periods. The estimated parametric coefficients and their significance (coefficients for Species, SL, Treatment and interaction between Treatment and Exposure Time) in the model are shown. The adjusted R^2 of the model with the random intercept fish ID and dependent variable RatioN was 0.36, and deviance explained by the ICC values was up to 28%. Significant p values for the explanatory variables such as fish identity were calculated using Satterthwaite d.f. The most appropriate parsimonious model was the most complex model, selected based on the AIC.

	Est	S.E.	t val.	d.f.	p
Intercept	-0.12	0.13	-0.89	184.93	0.37
Species (gibel carp)	0.08	0.02	4.45	117.10	0.00
SL	0.00	0.00	1.96	182.51	0.05
Treatment PE	-0.03	0.03	-0.85	300.65	0.40
Treatment TWPs	-0.00	0.03	-0.12	300.65	0.91
Treatment control Time 14	0.06	0.03	2.12	234.07	0.04
Treatment PE Time 14	0.05	0.03	1.82	235.39	0.07
Treatment TWPs Time 14	0.06	0.03	2.15	235.51	0.03
Treatment control Time 60	0.03	0.03	1.08	241.09	0.28
Treatment PE Time 60	0.06	0.03	2.28	244.44	0.02
Treatment TWPs Time 60	0.01	0.03	0.27	241.50	0.79

Table 3. Linear mixed effects model for the total activity in the open field test of gibel carp and crucian carp from the observations recorded for a group of 120 fishes, with 40 fishes each in the control and 14-day and 60-day exposure periods. The estimated parametric coefficients and their significance (coefficients for Species, SL, Treatment and interaction between Treatment and Exposure Time) in the model are shown. The adjusted R^2 of the model with the random intercept fish ID dependent variable TotalN was 0.18, and the deviance explained by the ICC values was up to 14%. Significant p values for the explanatory variables such as fish identity with respect to time were calculated using Satterthwaite d.f.

	Est	S.E.	t val.	d.f.	p
Intercept	6087.21	2783.74	2.19	165.27	0.03
Species (gibel carp)	588.99	378.98	1.55	116.40	0.12
SL	-1.27	29.40	-0.04	161.02	0.97
Treatment PE	-737.33	698.99	-1.05	335.18	0.29
Treatment TWPs	-760.97	699.00	-1.09	335.17	0.28
Treatment control Time 14	95.57	648.98	0.15	233.83	0.88
Treatment PE Time 14	-35.10	649.85	-0.05	234.87	0.96
Treatment TWPs Time 14	1684.65	649.93	2.59	234.97	0.01
Treatment control Time 60	253.28	653.64	0.39	239.42	0.70
Treatment PE Time 60	1043.68	655.89	1.59	242.10	0.11
Treatment TWPs Time 60	1546.91	653.91	2.37	239.74	0.02

Figures

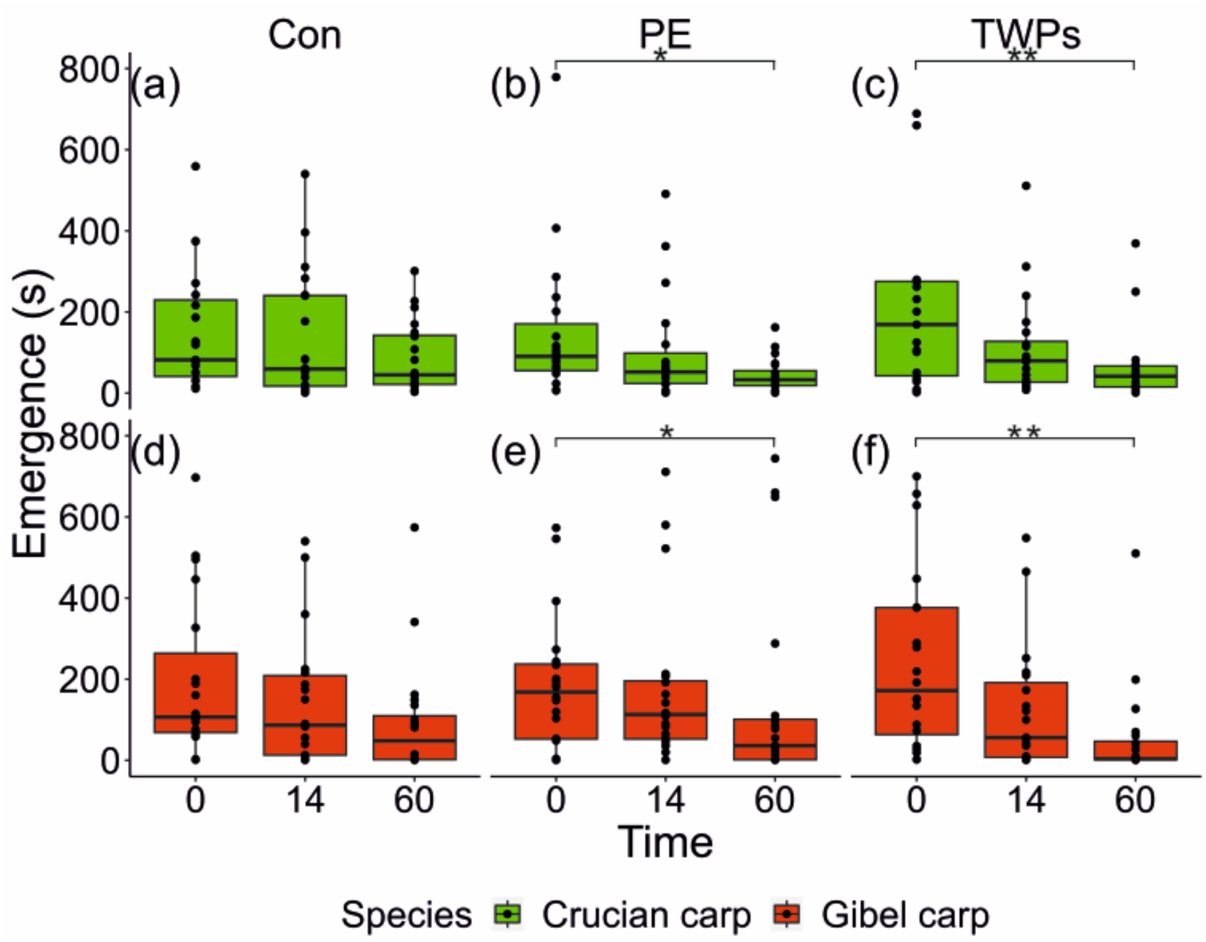

Figure 1. Emergence time (a proxy of fish boldness) differed between native crucian carp (*Carassius carassius*) and invasive gibel carp (*C. gibelio*) and declined with experimental duration (evaluated at 0, 14 and 60 days). PE treatment – 0.1% polyethylene microplastic food content, TWPs treatment – 0.1% tire wear particles food content, Control – no addition to the food pellets. Points = individual data; boxplots: thick line = median, box = 50% of interquartile range, whiskers = outer 25% of interquartile range excluding outliers.

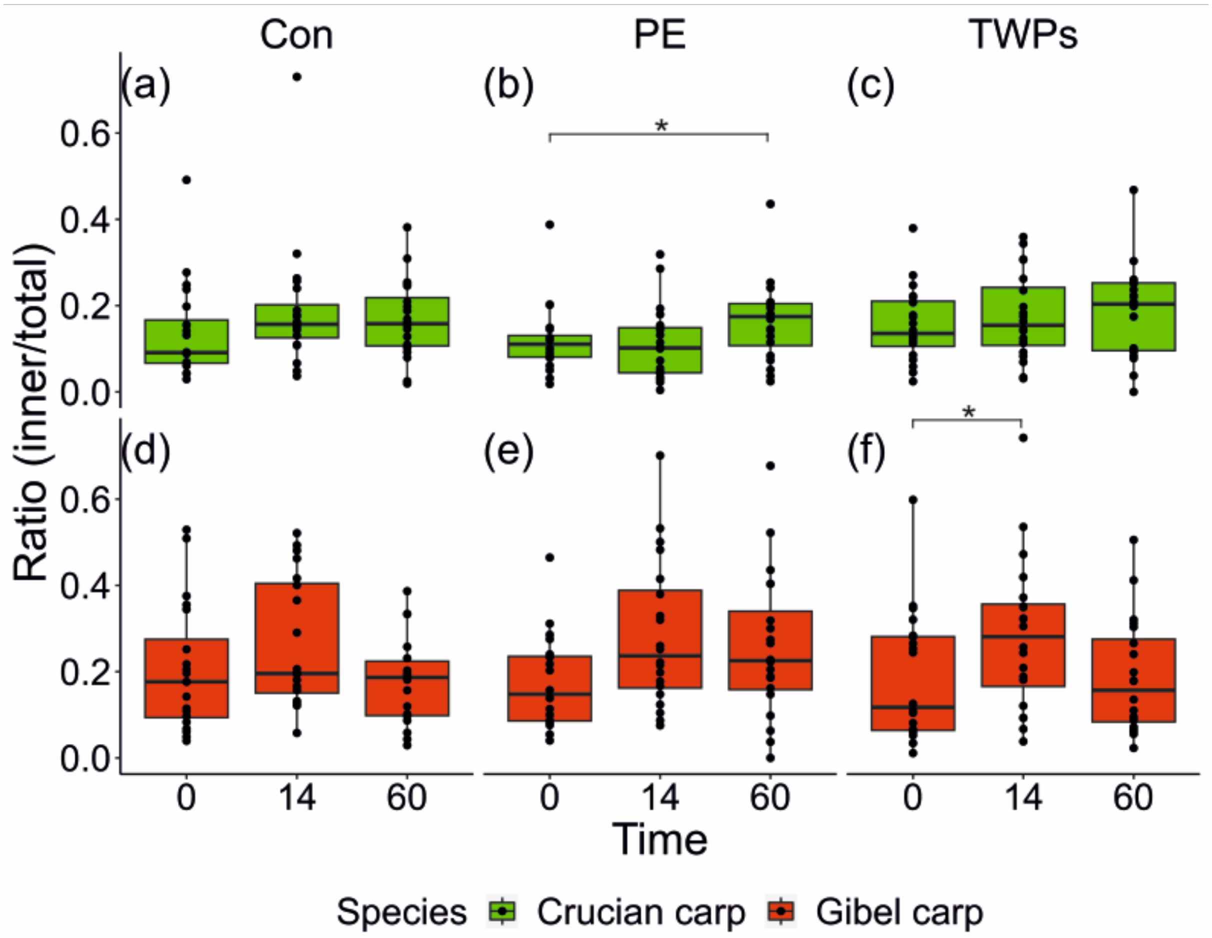

Figure 2. Ratio of time spent in the arena centre to the total test time of native crucian carp (*Carassius carassius*) and invasive gibel carp (*C. gibelio*) during exposure to the control, PE and TWPs treatments. The native species were more prone to risk at the end of the PE treatment. PE treatment – 0.1% polyethylene microplastics food content, TWPs treatment – 0.1% tire wear particles food content, Control – no addition to the food pellets. Points = individual data; boxplots: thick line = median, box = 50% of interquartile range, whiskers = outer 25% of interquartile range excluding outliers.

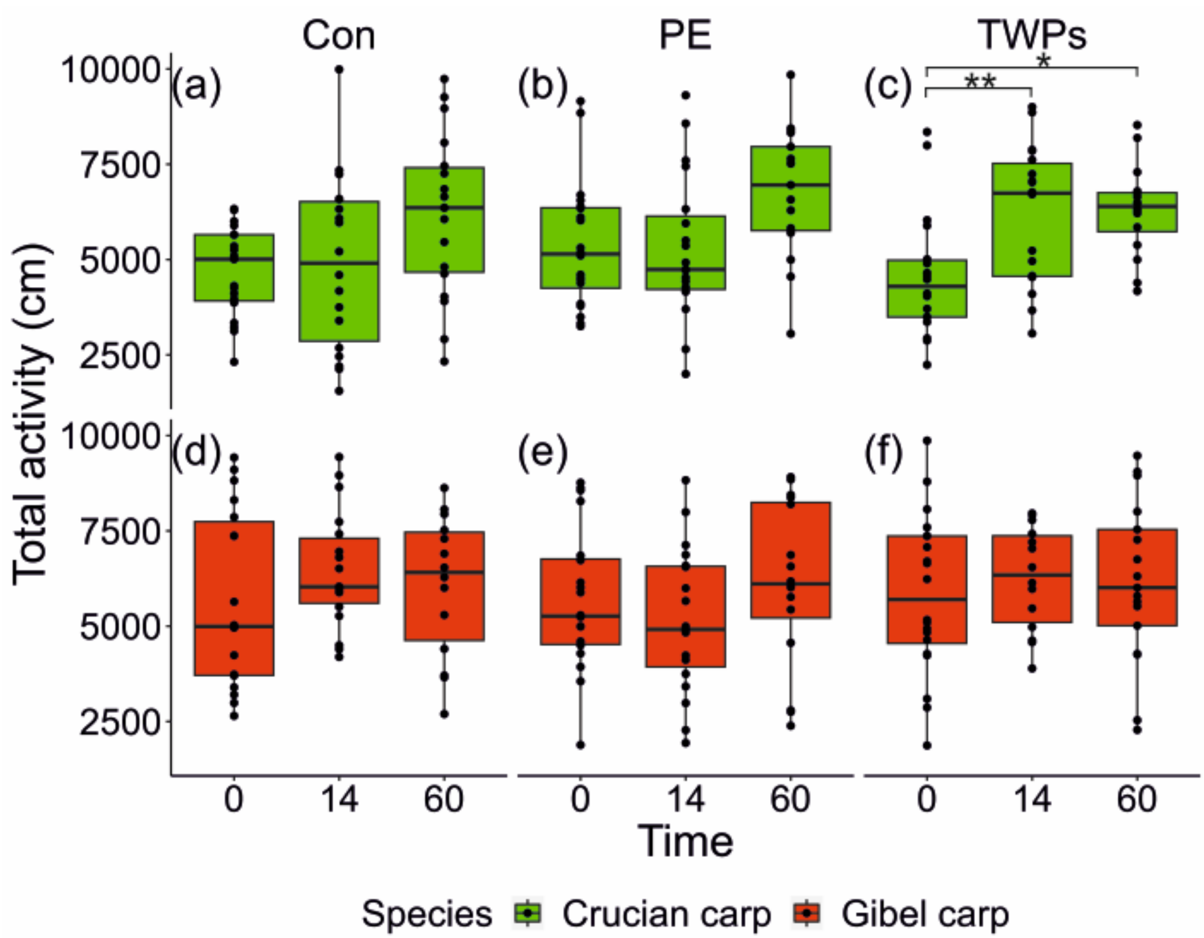

Figure 3. Total activity of native crucian carp (*Carassius carassius*) and invasive gibel carp (*C. gibelio*) represented as the total distance swam in cm during the behavioural trials. The invasive species were more locomotive than the native species in all the treatments. PE treatment – 0.1% polyethylene microplastics food content, TWPs treatment – 0.1% tire wear particles food content, Control – no addition to the food pellets. Points = individual data; boxplots: thick line = median, box = 50% of interquartile range, whiskers = outer 25% of interquartile range excluding outliers.

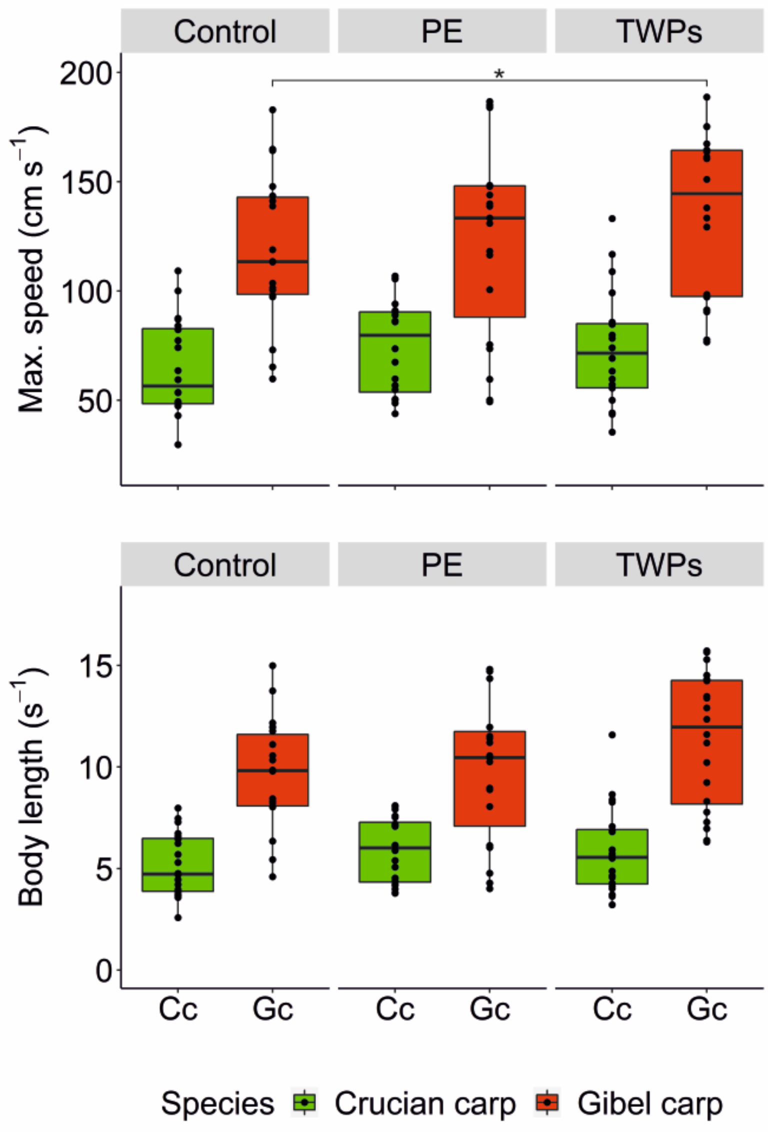

Figure 4. Swimming activity of native crucian carp (*Carassius carassius*) and invasive gibel carp (*C. gibelio*) represented as the total distance swam in cm s^{-1} at the end of the 60-day experimental period. The invasive species had better swimming performance than the native species in all the treatments. PE treatment – 0.1% polyethylene microplastics food content, TWPs treatment – 0.1% tire wear particles food content, Control – no addition to the food pellets. Points = individual data; boxplots: thick line = median, box = 50% of interquartile range, whiskers = outer 25% of interquartile range excluding outliers.

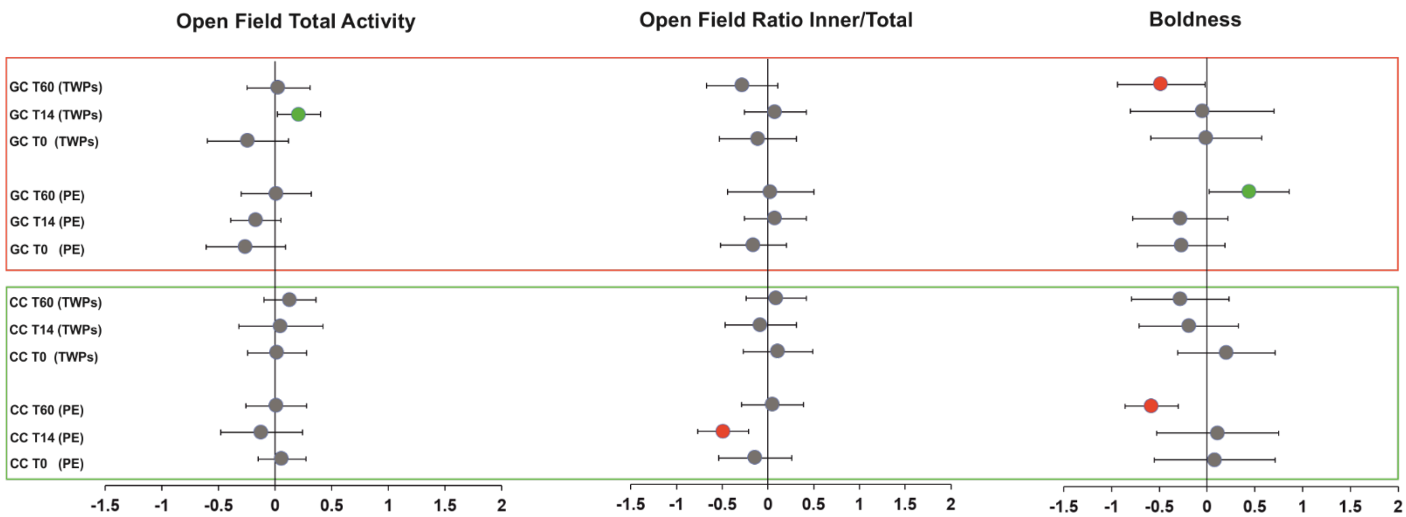

Figure 5. Forest plot showing cumulative impacts the native *Carassius carassius* (CC) and the invasive *C. gibelio* (GC) due to different treatments, PE and TWPs (ln–response ratio; mean ±95% confidence intervals). Outer rectangular red and green lines around the forest plots indicate a distinction between the invasive gibel carp (on the top) and native crucian carp (at the bottom). Red circles: negative ratios; green circles: positive ratios; grey circles: non-significant ratios, across time 0, 14 and 60 days. Cumulative effects are significant if confidence intervals do not overlap zero.