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The invasive red swamp crayfish (*Procambarus clarkii*) as a bioindicator of microplastic pollution: Insights from Lake Candia (northwestern Italy)

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A R T I C L E I N F O

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A B S T R A C T

Microplastic pollution has become pervasive. Identifying a bioindicator species to track the occurrence and effects of microplastics (MPs) on ecosystems is crucial for determining their impact on the environment. The digestive tract of *Procambarus clarkii* was thought to be of interest for investigating MPs accumulation in freshwater organisms. Our hypothesis was that the same type of MPs found in abiotic compartments (water and sediment) could be found in *P. clarkii*, which would make it an ideal candidate for use as a bioindicator of MP pollution in freshwater ecosystems. Water, sediment, and *P. clarkii* specimens were collected from four sites in a lentic ecosystem (Lake Candia; northwestern Italy) for two consecutive years (2021–2022). The mean MPs abundance was 1.75 ± 0.95 items/m^3 in 2021 and 2 ± 0.81 items/m^3 in 2022 in the water samples and 6.75 ± 1.5 items/kg and 8 ± 0.81 items/kg in the sediment samples in 2021 and 2022, respectively. In 2021, the average was 0.06 ± 0.07 items/g in the males and 0.05 ± 0.05 items/g in the females; in 2022, the average was 0.04 ± 0.05 items/g and 0.05 ± 0.06 items/g in the males and the females, respectively. MP fibers and fragments (black, white, blue, light blue) of polypropylene and polyethylene terephthalate were found in the biotic and the abiotic compartments. The generalized linear mixed model revealed that the number of items/g was predicted only by total weight: the lowest number of items/g was recorded for crayfish with the highest weight probably due to the feeding habits of *P. clarkii*. Our findings suggest that the invasive *P. clarkii* (smaller individuals, in particular) could be a good candidate bioindicator for MP pollution since the same type of MP items were recorded in the abiotic compartments. Further research is needed to better understand the feeding behavior of *P. clarkii* and the dynamics of MPs in aquatic ecosystems.

1. Introduction

The modern era of human history is considered the Plastic Age (Thompson et al., 2009). About 400 million tons of plastic are produced annually and production is projected to double by 2050 (PlasticsEurope, 2022). Worldwide plastic production has increased with its ever-wider range of use (medical, health, technological, food) and application by virtue of its strength, flexibility, low cost, and waterproofness (Anagnosti et al., 2021). The world annual production of plastics was 1.7 million tons in the 1950 s, which rose to 390.7 million tons in 2021, 57.2 million tons (14.64%) of which produced in Europe alone (PlasticsEurope, 2022). Only 10.1% of plastic waste is recycled, 12% is incinerated, and 79% ends up in discharges (PlasticsEurope, 2022). The improper management and disposal of plastic materials have led to the accumulation of plastic waste in the environment, where it has become a pollutant of worldwide concern (Zhang et al., 2022).

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Plastic is also extensively used in agriculture in the form of plastic films for mulching, irrigation with wastewater and fertilization with biosolids, which number among the main sources of plastic debris in terrestrial ecosystems (Tian et al., 2022). The soil and agricultural sediments are a major environmental reservoir of plastic debris (Zhang et al., 2022).

Plastic waste breaks down into micro- and nano-plastics that pose a threat to ecosystem health (Multisanti et al., 2022). Microplastics (MPs) refers to solid plastic forms released into the environment and varying in size (1–5000 μm), shape, density, color, and polymer types (Frias and Nash, 2019). Generally, MPs are classified as primary or secondary based on their origin (Jaikumar et al., 2019). Primary MPs are microscopic in size, directly released into the environment, and usually associated with personal care products (Issac and Kandasubramanian, 2021). Secondary MPs refer to fragments from degradable macro-scale plastic items before they enter the environment via human activity and environmental stressors (Issac and Kandasubramanian, 2021).

Because of their slow degradation and small size, MP particles are ingested by several animal species, transported in the organism itself and throughout trophic webs, ultimately resulting in bioaccumulation (Wang et al., 2021a; Hodkovicova et al., 2022; Malli et al., 2022). Aquatic organisms, including plankton, invertebrates, and vertebrates, ingest MPs (Fu et al., 2020; Rodrigues et al., 2021; Bertoli et al., 2022; Doyle et al., 2022). Microplastic particles can accumulate in the digestive tract of aquatic organisms or be transported to other tissues and organs by the circulatory system (Impellitteri et al., 2022).

To date, most MPs research has focused on the marine environment, as the oceans are considered the main basin of contamination (Amelia et al., 2021). However, since most plastic is used and disposed of on land, both terrestrial and freshwater environments may be affected by MP pollution and serve as long-term reservoirs (Wang et al., 2021b). Recent monitoring studies have established that MPs are ubiquitous in biotic and abiotic compartments of freshwater watercourses and lakes (Talbot and Chang, 2022). Globally, MPs are detected at very high concentrations in rivers and lakes (Dusaucy et al., 2021). Identifying sentinel (bioindicator) species to track MPs occurrence and effects on ecosystems is essential for determining their environmental impact and for developing management strategies (Piccardo et al., 2021; Multisanti et al., 2022; Martyniuk et al., 2023). Despite their ecological and economic importance, freshwater decapod crustaceans and their interaction with MPs have been understudied (D'Costa, 2022; Yin et al., 2022). Recent studies have suggested that decapods may be able to fragment MPs into nano-sized particles by digestion and that nanoplastics can alter their survival, growth, nutrition and energy metabolism, immunity, and antioxidant defense (Capanni et al., 2021).

The red swamp crayfish *Procambarus clarkii* (Girard, 1852) is a widely distributed freshwater benthic crustacean (Gherardi, 2006; Manfrin et al., 2019). Originally from Mexico and South-Central America, it was captured in the 1950s and successfully translocated worldwide for aquaculture (Gherardi, 2006). It is ranked by the European Union in the top places among the 100 most harmful “Invasive Alien Species” [Regulation (EU) 1143/2014] (Souty-Grosset et al., 2016). Indeed, in food webs *P. clarkii* acts as keystone species, causing a significant decrease in biomass and a decline in biodiversity, with unfavorable ecological and economic impacts (Dörr et al., 2020). Because of its biological and behavioral plasticity and dispersal patterns, it can colonize a wide range of environments, lagoon and brackish ecosystems included (Dörr et al., 2020). This invasive species that can tolerate highly polluted environments and has served as a bioindicator of environmental pollution by heavy metals, cyanotoxins, and organic compounds (Tricarico et al., 2008; Faria et al., 2010; Goretti et al., 2016; Mistri et al., 2020). Being an opportunistic benthic feeder, it is susceptible to plastic ingestion (Capanni et al., 2021). Thus, *P. clarkii* could represent a good potential candidate to investigate the occurrence and temporal fluctuations of MPs in many aquatic environments. Freshwater crayfish are also a primary source of animal protein (Haubrock et al., 2021). In China, for example, crayfish are grown or co-cultured in rice paddy ponds (Lv et al., 2019). Elevated amounts of MPs have been found in pond water and sediment of shrimp farms and fields in co-culture. In particular, MPs have been detected in various tissues of *P. clarkii*, with greater accumulation in the digestive tract (Zhang et al., 2021). We thought it of interest to study MPs accumulation in the digestive tract of *P. clarkii* and the factors (i.e., biometrical features) influencing MPs accumulation. Our hypothesis was that since the same types of MPs can be found in *P. clarkii* and abiotic compartments (water and sediment), it would be a good candidate bioindicator of MP pollution in lentic freshwater ecosystems. To do this, we sampled water, sediment, and specimens of *P. clarkii* for two consecutive years (2021–2022) in a small lake (Lake Candia) located in northwestern Italy.

2. Material and methods

2.1. Study area

Lake Candia is an important wetland in Piedmont (northwest Italy; 45°19′28″N, 7°54′35″E) (Fig. 1). Compared to other lake basins in Piedmont, it has retained its naturalness. Located near the Western Alps, the Lake Candia Nature Park is a listed biotope in Piedmont and classified as a Site of Community Importance (SCI-IT1110036) under the European Union's Habitats Directive (92/43/EEC).

The Reserve comprises two wetlands, a glacial lake and a marsh connected by a channel system measuring 3 km^2 in area. The lake lies at an altitude of 226 a.s.L., measures 1.52 km^2 in area and 5.5 km in perimeter (Fig. 1). The average depth is about −5 m, the maximum depth roughly −7 m. The marsh measures 0.4 km^2 in area and consists of channels and shallow waters (≤−0.70 m). It is not fed by tributary streams but rather by underwater springs along its southern shore. The water turnover time is estimated at around 6–7 years.

The lake hosts several species. *Najas marina*, *Nymphoides peltata*, *Trapa natans*, and *Myriophyllum spicatum* represent the main macrophyte species. More than 200 bird species, amphibians (i.e., *Bufo bufo*, *Rana dalmatina*), reptiles (i.e., *Natrix natrix*), and fish (i.e., *Esox* spp.) are recorded in this lake (Donato et al., 2018). Furthermore, Donato et al. (2018) reported the presence of an established and reproductively active population of *P. clarkii*.

2.2. Water and sediment sampling

Water and sediment samples were collected in August 2021 and August 2022 to determine and quantify the MPs in the abiotic compartments of Lake Candia. Sediment samples (n = 3 replicates per site/year) were collected with a manual corer (250 cm^2 sampling surface) at four sampling sites (Fig. 1a-d). The samples were collected from the riverbed, a few meters from the shore, depending on accessibility and setting the nets for crayfish sampling (section 2.3). Sediment replicates collected from each site were then stored separately in stainless steel containers covered with aluminum foil to prevent external particle contamination and frozen at −20 °C until analysis (Pastorino et al., 2021; Bertoli et al., 2022). The samples were processed for MPs analysis (section 2.4). Water samples were collected at the same time as the sediment samples. An Apstein plankton net (opening 400 × 1000 mm; mesh size: 50 μm) hauled by a dinghy at low speed was used to determine the horizontal and vertical distribution and composition of MPs at each sampling site (Prata et al., 2019). The water samples (n = 3 replicates per site/year) were placed into glass jars (1 L), brought to the laboratory, and stored at +4 °C until MPs analyses. The net was cleaned between each replicate with ultrapure water to prevent contamination (Prata et al., 2019).

2.3. Crayfish sampling and dissection

A total of 90 specimens (n = 45 per year) of *P. clarkii* were captured

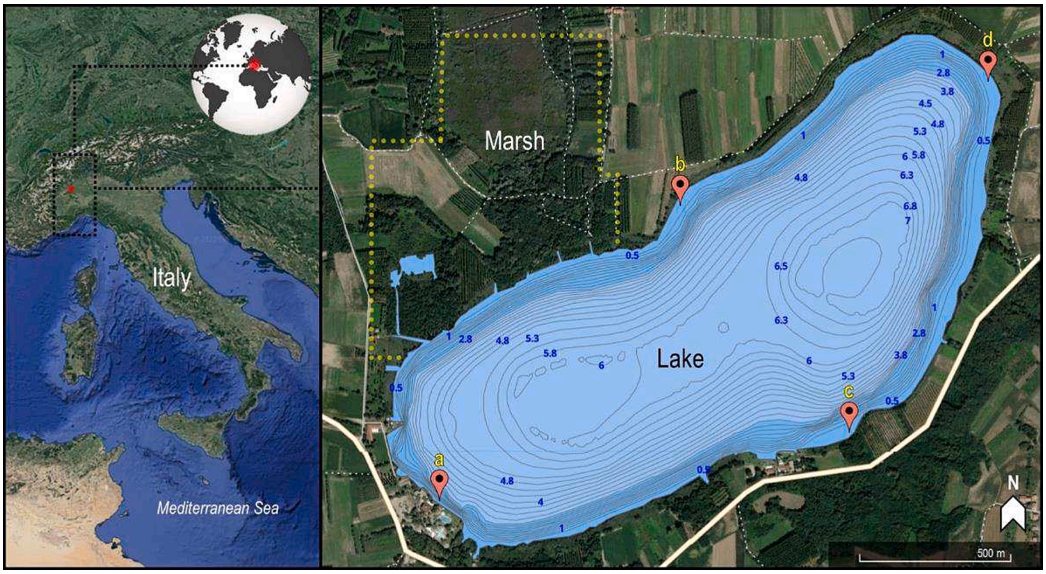

Fig. 1. Study area (marsh and lake) and sampling sites (site a 45°19′12″N, 7°54′02″E; site b 45°19′41″N, 7°54′35″E; site c 45°19′53″N, 7°55′18″E; site d 45°19′18″N, 7°54′59″E). The images were modified from Google Earth (left) and https://fishingapp.gpsnauticalcharts.com/ (right).

at the four sampling sites (Fig. 1). The crayfish were caught using a cylindrical crayfish creel trap (90 cm in length, 1 cm mesh size, 30 cm cross-section, and two funnels 10 cm in diameter) with bait (canned cat food). The traps were placed at mid-depth (0.5–2 m) at the four sampling sites and recovered 24 h later. Crayfish were removed and transported to the laboratory according to current regulations. They were placed in a dry, refrigerated steel container (+15 °C) to prevent contamination. On arrival at the laboratory, the crayfish were suppressed by hypothermia (refrigeration at 4 °C for at least 24 h then frozen at −20 °C for at least 1 week) without unnecessary suffering. The transport containers were disinfected, and the total number of catches recorded at each sampling site. In the laboratory, the crayfish were sexed, measured for cephalothorax length (CTL, from the tip of the rostrum to the posterior portion of the cephalothorax) using a 0.1 mm precision caliper (DCLA-0805, Vinca, China) and weighed using a 0.01 g precision digital balance (Radwag, PS06.R2, Poland). The crayfish were dissected with a stainless-steel dissecting kit (scalpel, forceps, scissors). The digestive tract was removed, weighed, wrapped in aluminum foil, and stored at −20 °C until MPs determination (section 2.4).

2.4. Microplastics determination

The sediment samples were extracted from a prefiltered saturated NaCl solution by mechanical agitation (20 min, 100 rpm); the supernatant was filtered through 6 μm pore paper disk filters (Whatman®, Sigma-Aldrich, St. Louis, MO, USA). The filters were placed on a glass Petri disk to prevent contamination during oven drying (35 °C). The dried samples were analyzed under stereomicroscopy, and the MP particles collected for chemical analysis. The digestive tracts were digested in a saturated solution of KOH + NaOH sonicated at 40 Hz for 20 min (30 °C), then filtered through a vacuum pump on a paper fiber filter disk (6 μm; Millipore®) and oven dried at 40 °C till complete exsiccation. The filters were sorted under stereomicroscopy (Nikon, P-DSL32, 10-80x) connected to a webcam (Nikon; P-DSL32, NS-Elemens D.4.60 64-bit). Targeted items were collected and analyzed by the μFT-IR technique (Thermo Fischer®, Nicolet iN10) in reflection mode. The target-item spectral match (%) was determined and compared to the spectral libraries of normal and aged microplastics (OMNICTM PictaTM software libraries, Thermo Fisher Scientific) integrated with spectral libraries from the BsRC laboratory. The limit of detection (LOD) of particle size was 10 μm. The MP particles were classified by shape and color according to Joint Research Centre criteria (2014).

2.5. Quality Assurance/Quality control

The QA/QC approach was taken to ensure that the data met the quality criteria (sample processing and storage, laboratory preparation, clean air conditions, target component, sample treatment, polymer identification) reported in the literature to ensure detection of ingested MPs in biota (Hermsen et al., 2018). Samples and sorting activities were performed under a box-glove (Iteco Engineering, mod. SGS20-13599, serial number 103421) to exclude sample pollution. Positive and negative controls (n = 5) were prepared to prevent respectively recoveries, airborne pollution, and cross contamination of samples. Negative controls resulted unpolluted, and the particle recovery rate was nearly 100% of spiking MPs items used. Instrument qualification was checked with standard reference materials (Thermo®, Instrument Qualification Kit).

2.6. Statistical analysis

Data normality and homoscedasticity were assessed using the Shapiro-Wilk and the Levene test, respectively. As the data were not normally distributed, the Mann-Whitney *U* test was used to check for differences in MPs abundance in abiotic and biotic compartments between the two sampling years. The Mann-Whitney *U* test was used to determine differences in biometric features (cephalothorax length and total weight) of crayfish sampled in 2021 and 2022 and sex (male and female). Pearson's correlation test (*cor.test* R-function) was performed to test for a correlation between the biometric features of the crayfish (cephalothorax length and total weight) and the features of the MPs accumulated in in their digestive tract (MPs size and number of items/g). A generalized linear mixed model (GLMM; *glmmTMB* R-package; Brooks et al., 2017; R Core Team, 2021) was run to compare the factors for the number of items/g in the digestive tract and the number of items/g as the response variable. As the residuals were non-normally distributed, we chose a beta distribution after fitting the model using the R-*fitdist* function (Delignette-Muller and Dutang, 2015). We used sex (male/female), total weight, MPs size, plastic chemical type (PET/PP), shape (fiber/fragment), and color (white/blue/light blue/black) as the fixed factors. The sampling year and the MPs size were the random factors. No collinearity was found between the fixed factors (range

VIFmi*N* 1.21; VIFmax 2.62; *check_collinearity* R-function; *performance* R-package). The likelihood ratio test (ANOVA with argument test Chisq; Dobson 2002) was used to verify the significance of the full model against the null model comprising only the random factors (Forstmeier and Schielzeth, 2011). The p-value for each predictor was calculated based on the likelihood ratio tests between the full and the null model by using the R-function *drop1* (Barr et al., 2013). Statistical significance was set at p-value < 0.05.

3. Results

3.1. Microplastics in water and sediment samples

Microplastics were detected in both water (cumulatively 7 and 8 items in 2021 and 2022, respectively) and sediment samples (cumulatively 27 and 32 items in 2021 and 2022, respectively) (Fig. 2). The mean MPs count (counts merged from the four sites) was 1.75 ± 0.95 items/m^3 in 2021 and 2 ± 0.81 items/m^3 in 2022 in the water samples and 6.75 ± 1.5 items/kg and 8 ± 0.81 items/kg in 2021 and 2022, respectively in the sediment samples (counts merged from the four sites). No statistically significant difference in MPs count in the water (Mann-Whitney *U* test, U = 6.5; p = 0.91) or the sediment (Mann-Whitney *U* test, U = 4; p = 0.31) samples between the two years was found. The mean size of the MP items (size merged for two years) was 623 ± 220 µm (range 135–830 µm) in the water samples and 823 ± 220 µm (range 150–1505 µm) in the sediment samples. Polypropylene (PP) and polyethylene terephthalate (PET) MPs were found in the water (2021: 57.1% PP, 42.9% PET; 2022: 62% PP, 38% PET) and the sediment (2021: 59.3% PP, 40.7% PET; 2022: 62% PP, 38% PET) samples (Fig. 2). Fibers (2021: 57 %; 2022: 62.5%) and fragments (2021: 43; 2022: 37.5%) were recorded in both compartments. Black (43% and 50% in 2021 and 2022, respectively), white (28.5% and 37.5% in 2021 and 2022, respectively), and blue (28.5% and 12.5% in 2021 and 2022, respectively) were recorded in the water samples, while black (44.4% and 46.9% in 2021 and 2022, respectively), white (26% and 28.1% in 2021 and 2022, respectively), blue (18.5% and 18.7% in 2021 and 2022, respectively), and light blue (11.1% and 6.3% in 2021 and 2022, respectively) were recorded in the sediment samples.

3.2. Biometric features of crayfishes

A total of 21 males and 24 females and 23 males and 22 females were captured in 2021 and 2022, respectively. The sex ratio was nearly identical for the two years (2021, 47% male, 53 % female; 2022, 51% male, 49 % female). No statistically significant difference in cephalothorax length or total weight between males and females was found (Mann-Whitney *U* test, p greater than 0.05 for both samplings). The mean cephalothorax length (male and female merged) was 8.80 ± 1.13 cm in 2021 and 8.46 ± 0.95 cm in 2022. The mean total weight (male and female merged) was 16.06 ± 5.67 g in 2021 and 15.92 ± 5.66 g in 2022.

3.3. Microplastics accumulation in crayfish

The digestive tract of the males contained 0.06 ± 0.07 items/g on average (0.83 ± 0.76 items/individual) in 2021, while that of the females contained on average 0.05 ± 0.05 items/g (0.88 ± 0.75 items/individual). MPs accumulation was similar for 2022: an average of 0.04 ± 0.05 items/g (0.65 ± 0.74 items/individual) and 0.05 ± 0.06 items/g (0.67 ± 0.77 items/individual) for males and females, respectively. There was no statistically significant difference in items/g between males and females for both sampling years (Mann-Whitney *U* test, p greater than 0.05) (Fig. 3).

Polypropylene (PP) and polyethylene terephthalate (PET) were the only MPs chemical types found in the samples from both years (2021, 72% PP, 28% PET; 2022, 67% PP, 33% PET; sex merged). An example of MP item and relative µFT-IR spectrum is reported in Figure S1 (Supplementary Material).

Black (34% and 38% in 2021 and 2022, respectively), white (32% and 33% in 2021 and 2022, respectively), blue (16% and 23% in 2021 and 2022, respectively), and light blue (18% and 6% in 2021 and 2022, respectively).

When we tested the correlation between total weight and cephalothorax length, we found that these proxies of body size correlated positively with each other (Pearson's correlation, r = 0.692, t = 5.414, df = 32, p $<$ 0.001). We also found a significant negative correlation between cephalothorax length/total weight and the number of items/g (no. items/g *vs.* total weight, t = −5.334, df = 32, p $<$ 0.001; no. items/g *vs.* cephalothorax length, t = −2.682, df = 32, p = 0.01). No statistically significant correlation for any of the other parameters was found (Fig. 4).

When we investigated which factors could predict the number of items/g in the digestive tract, we found that the full model differed from the null model significantly (χ^2 = 35.490, df = 12, p $<$ 0.001). The number of items/g was significantly predicted only by the total weight: the crayfish highest in weight had the fewest items/g (Table 1; Fig. 5). None of the other factors reached statistical significance.

4. Discussion

Despite differences in sampling and MPs determination methods, the MPs in the water samples from Lake Candia were similar to those

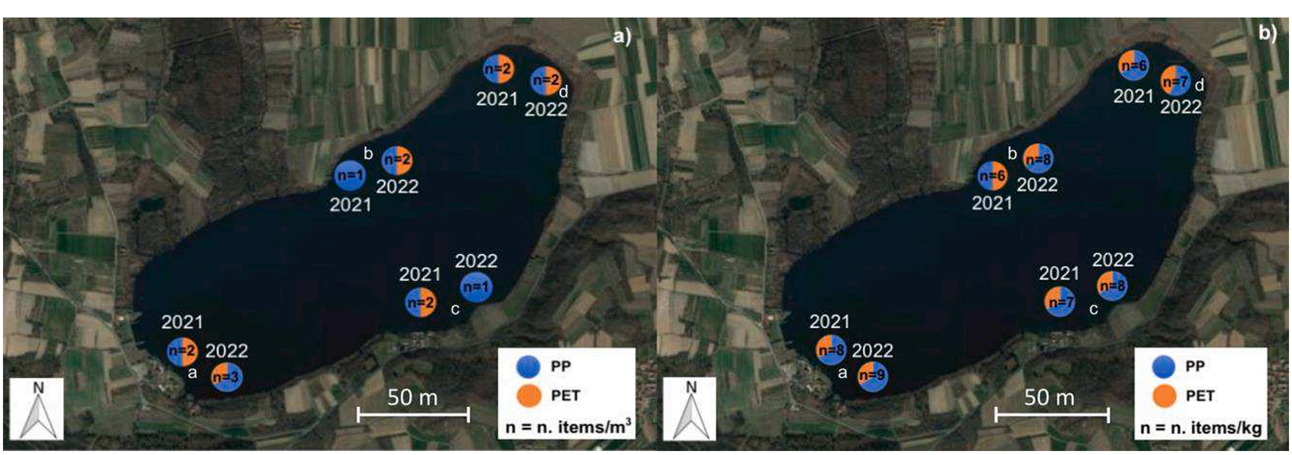

Fig. 2. Microplastic items detected in water (a) and sediment (b) samples in Lake Candia in 2021 and 2022. The pie charts report the chemical type of microplastic items recorded at each sampling site.

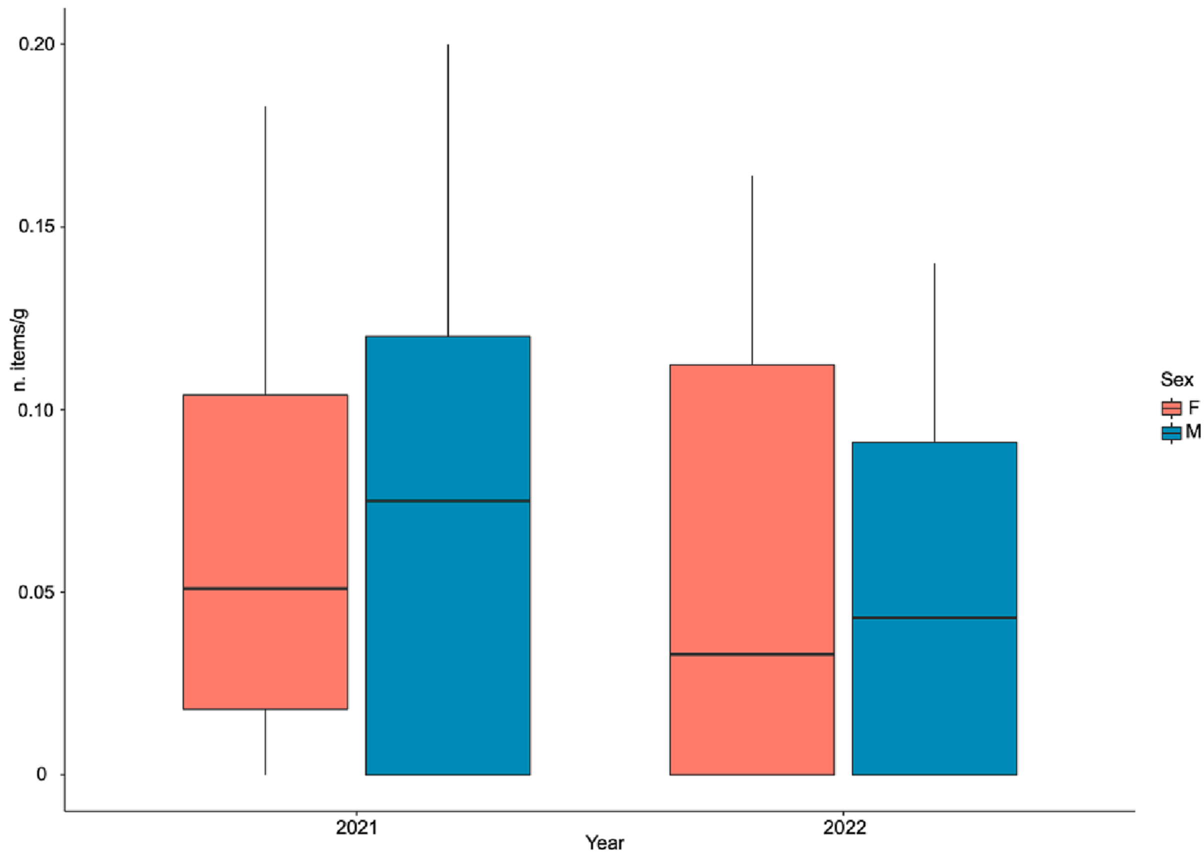

Fig. 3. Boxplots of microplastic items (no. of items/g of digestive tract) in male and female *Procambarus clarkii* sampled in 2021 and 2022.

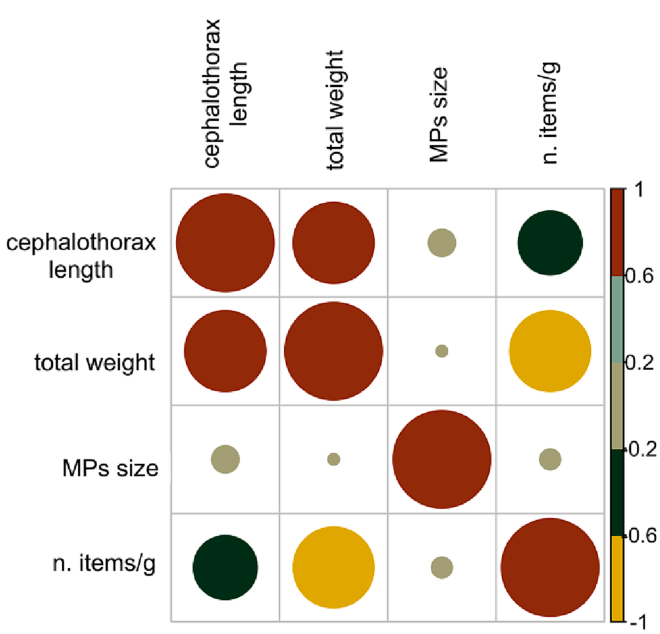

Fig. 4. Results of Pearson's correlation test for cephalothorax length and total weight, microplastic size, and number of items/g.

recorded for other shallow lakes in Italy (i.e., Lake Chiusi, 5.8 maximum depth; 2.68–3.36 particles/m^3) (Fischer et al., 2016). Similar values were also recorded for larger deep lakes in Central Italy (Lake Bolsena, 0.21–4.08 items/m^3; Lake Bracciano 2.4 items/m^3; Fischer et al., 2016; Cera et al., 2022, respectively). Differently, the amount of MPs in the water samples from Lake Candia was lower than that recorded for larger

Table 1
Generalized linear mixed model for number of microplastic items/g recorded in *Procambarus clarkii* and microplastic/crayfish features. "a" not shown because did not have a meaningful interpretation. Statistically significant p-values are given in bold.

	Estimate	SE	DF	z-value	p-value
(Intercept)	$-5.833e^{-01}$	$3.467e^{-01}$	a	−1.682	a
Sex	$-3.131e^{-02}$	$1.079e^{-01}$	1	−0.290	0.774
Total weight	$-8.976e^{-02}$	$1.193e^{-02}$	1	−7.522	**<0.001**
MP length	$3.893e^{-05}$	$1.517e^{-04}$	1	0.257	0.778
MP type (PP)	$-1.436e^{-01}$	$1.444e^{-01}$	1	−0.994	0.332
MP form (Fragment)	$-9.743e^{-02}$	$1.099e^{-01}$	1	−0.886	0.376
MP color (Blue)	$1.852e^{-02}$	$1.498e^{-01}$	3	0.124	0.107
MP color (Light blue)	$3.308e^{-01}$	$1.556e^{-01}$	3	2.127	a
MP color (Black)	$-1.214e^{-01}$	$1.343e^{-01}$	3	−0.904	a

and deeper lakes in Europe: Lake Brienz, 36,000 items/km^2 and Lake Maggiore and Lake Geneva, 220,000 items/km^2 (Faure et al., 2015). The amount of MPs in the sediment samples from Lake Candia was lower than that recorded for Lake Bolsena (112 items/kg d.w.) and Lake Chiusi (234 items/kg d.w.). Based on lake depth and area, the MPs in sediment ranged from 0.7 items/kg to 7707 items/kg, with a median value of 385 items/kg and lower amounts recorded for rural, remote lakes compared to urban lakes (Dusaucy et al., 2021).

Microplastics in lakes come from a variety of sources and can be transferred from one source to another (Dusaucy et al., 2021; Yang et al., 2022). The three main sources of plastic pollution are: macroplastic fragmentation in the environment, wear and tear of products containing plastic polymers (textile fiber fragmentation during clothes washing, paints, tires), and intentional origin (Dusaucy et al., 2021). Although classified as a protected area, Lake Candia was subject to anthropogenic impact until the late 1980s when lake discharges were diverted, and

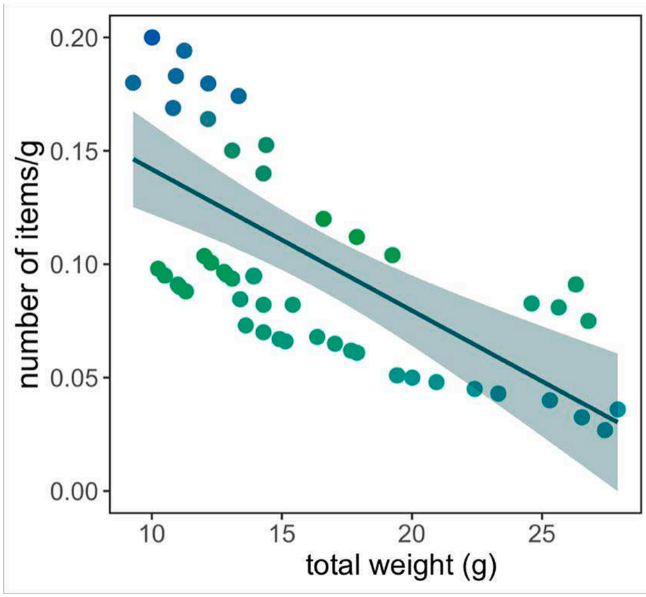

Fig. 5. Relationship between total weight (g) of *Procambarus clarkii* and number of microplastic items/g in the digestive tract.

wastewater treatment plants constructed. Nonetheless, many other factors may be responsible for the MPs pollution of Lake Candia.

Previous research has shown that climate change influences MPs in aquatic ecosystems (Burgos-Aceves et al., 2022). For example, MPs emission and transport increase with precipitation, flooding, glacial melting, and runoff; atmospheric plastic levels rise with strong winds; MPs suspension from sediments is greater due to wind and water currents and temperature changes in water; plastic retention increases due to evaporation of lake water. Some sources of MPs in Lake Candia could derive in part from sewage system malfunction caused by prolonged rain, discharge of excess runoff through overflows, and from the washout of the hydrographic basin and rainfall.

The MPs size in the abiotic compartments of Lake Candia was comparable to the range reported in the literature. Most plastic items recorded in freshwater lakes are <2 mm (Dusaucy et al., 2021), except for lakes in the Siling Co basin (northern Tibet) where MPs size ranges from 1 to 5 mm (Zhang et al., 2016).

Polymeric identification of MP items revealed only two polymer types in the abiotic compartments of Lake Candia. These findings are shared by previous studies that reported that polypropylene (PP) and polyethylene (PE) alone account for more than half of the main MPs contaminants in freshwater ecosystems worldwide, followed by polyethylene terephthalate (PET) and polystyrene (PS) (Cera et al., 2020). In Europe, the proportion of PP in water is 50.0%, followed by PE (15.5%), PET (1.3%), PS (27.3%), PVC (0.2%), and others (5.8%), whereas PA (79.9%) makes up the major type of MPs in the sediment, followed by PS (10.0%), PP (0.1%), and others (10%) (Yang et al., 2022). Six polymer classes dominate the plastic materials market and constitute 81% of global production: polyethylene (PE, high and low density), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS, including expanded polystyrene), polyurethane (PUR), and polyethylene terephthalate (PET) (PlasticsEurope, 2022).

MPs fibers and fragments were recorded in the surface water and the sediment samples from Lake Candia; they are the most frequently identified polymer forms in freshwater lakes worldwide (Dusaucy et al., 2021). Fibers appear to be land-based MPs; sewage from clothes washing is the main source of fiber (Browne et al., 2011; Schirinzi et al., 2020). Since rowing competitions are held on Lake Candia, the fibers could derive from athlete sportswear. Furthermore, because the primary source of MPs is the fragmentation of larger plastic items, fragments account for a major portion of the total number of MPs in freshwater systems worldwide (Yang et al., 2022).

Since nearly the same shape, color, and chemical type of MPs were recorded in the *P. clarkii* specimens and in the abiotic compartment, this species could be considered a good bioindicator of MP pollution. To date, only two studies have assessed MP occurrence in this species. Zhang et al. (2021) studied the occurrence and distribution of MPs in *P. clarkii* from pond and rice-crayfish co-culture breeding modes in Jianli Prefecture (China). They found on average MPs abundance from 0.75 ± 0.13 to 0.92 ± 0.19 items/individual, with slightly higher values (2.5 ± 0.6 items/individual) recorded in *P. clarkii* captured in three rice-fish culture stations in Shanghai (China) (Lv et al., 2019).

We found an inverse relationship between MPs (items/ g of digestive tract) and crayfish weight. Despite its opportunistic feeding habits, *P. clarkii* is a polytrophic feeder that selectively feeds on macrophytes and macroinvertebrates (Correia, 2003). It displays an ontogenetic dietary shift, with juveniles feeding more intensively on aquatic macroinvertebrates and adults consuming more detritus and plant material (Correia, 2003). Aquatic macroinvertebrates are noted to accumulate high levels of MPs items compared to those found in water and sediment, especially in collector-gatherers that feed on material deposited on sediment (scavenging for dead organisms and other food particles) (Bertoli et al., 2022). The Chironomidae family, which is a key component of the macroinvertebrates inhabiting lentic ecosystems (Pastorino et al., 2020), can accumulate high MPs loads per gram wet weight (Akindele et al., 2020). Accordingly, it is plausible that juveniles feeding on macroinvertebrates contaminated by MPs will accumulate more MPs than adults. The MPs size (range, 134–1505 μm) found in the crayfish from Lake Candia was higher than the 50–500 μm recorded in *P. clarkii* from Hubei Province (China) (Zhang et al., 2021) bur lower than that measured in the water and sediment samples in the present study.

These small MPs produced mainly by the fragmentation of larger plastic debris can be easily and accidentally ingested by aquatic organisms during feeding (Roch et al., 2020). Fibers were mainly isolated from the digestive tract samples, as recorded for the abiotic compartments of Lake Candia. This finding is in line with previous studies that reported that fibers accounted for more than 90% of all items recovered from decapod crustaceans (Yin et al., 2022). Fiber aggregation within the digestive tract can increase retention time, ultimately affecting growth and development (Nan et al., 2020). Fibers have been found to predominate in a wide range of aquatic taxa (from bivalves to fishes), as well as in air and water (González-Pleiter et al., 2020). Black and white items were most often detected in the digestive tract of the *P. clarkii* from Lake Candia. Zhang et al. (2021) found that the stomach and the gut content of *P. clarkii* from Hubei Province (China) contained most often transparent fibers, followed by red and blue fibers. This could be attributed to the feeding habits of crayfish, as well as their prey characteristics. MPs with a color similar to the prey or those mixed with their food source(s) are likely to be ingested by aquatic organisms (Roch et al., 2020). Although found in other aquatic organisms, previous research reported that *Decapterus muroadsi* (Teleostei, Carangidae) preferred blue MPs similar to their blue copepod prey (Ory et al., 2017) and *Seriolella violacea* (Teleostei, Centrolophidae) preferred black MPs similar to food pellets (Ory et al., 2018) More research is needed to understand whether crayfish have MPs color preference. Finally, all MPs items were PP and PET, in line with their recovery in the abiotic compartments of Lake Candia, wide usage, and occurrence in crustacean decapods (D'Costa, 2022).

5. Conclusions

Based on their life-history strategies, a variety of invertebrates has been identified as a potentially good bioindicator of MP pollution in their respective habitats. To our best knowledge, this is the first study of *P. clarkii* as a bioindicator to assess MP pollution in lakes. We found that the number of items/g was predicted only by the specimen's total weight, with more MPs items found in smaller individuals. This suggests that sampling smaller crayfish for MPs will yield a greater chance to recover MPs items. Our hypothesis that the digestive tract content of *P. clarkii* would reflect the occurrence and type of MPs items in the study lake was supported by the finding of items having the same shape, color, and chemical type as recorded in the abiotic compartments. Summarizing, measuring MPs levels at the individual and the population level is a simple way to assess MPs bioavailability, an additional factor in ecological risk assessment. The use *P. clarkii* to monitor MP pollution could also strengthen strategies to contain or eradicate (if possible) non-native crayfish in protected areas like Lake Candia. Further studies are needed to better understand the transfer of MPs in the trophic web, and the feeding habits of *P. clarkii* in Lake Candia and other freshwater ecosystems.

CRediT authorship contribution statement

Paolo Pastorino: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Serena Anselmi:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Anna Zanoli:** Data curation, Investigation, Methodology, Writing – review & editing. **Giuseppe Esposito:** Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. **Fabio Bondavalli:** Investigation, Methodology, Writing – review & editing. **Alessandro Dondo:** Investigation, Methodology, Writing – review & editing. **Alessandra Pucci:** Investigation, Methodology, Writing – review & editing. **Elisabetta Pizzul:** Investigation, Methodology, Writing – review & editing. **Caterina Faggio:** Conceptualization, Methodology, Writing – review & editing. **Damià Barceló:** Conceptualization, Methodology, Writing – review & editing. **Monia Renzi:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Marino Prearo:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110200.

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