



## Bioplastics on marine sandy shores: Effects on the key species *Talitrus saltator* (Montagu, 1808)



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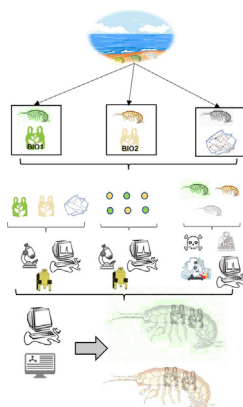
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### HIGHLIGHTS

- The ability of *T. saltator* to feed on bioplastics is confirmed.
- Analyses performed on bioplastics and faecal pellets showed structural differences.
- Different types of ingested bioplastics may have diverse effects on *T. saltator*.
- Supralittoral amphipods may play a role in the degradation of bioplastics in the environment.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Talitrid amphipods are an important component of detritus web, playing a key role in the fragmentation of organic matters of marine and terrestrial origin, and it is well known that sandhoppers ingest microplastics. To assess the effective consumption of bioplastics and their effects on survival rate and on pollutants transfer (i.e. phthalates) on supralittoral arthropods, laboratory experiments were conducted by feeding adult *T. saltator* with two different types of bioplastic commonly used in the production of shopping bags. Groups of about 20 individuals were fed with 10 × 10 cm sample sheets of the two types of bioplastic for four weeks. The results show that amphipods ingest bioplastics even in the absence of microbial film and that ingestion of bioplastic can have effects on talitrid amphipods. Microtomographic analyses of faecal pellets seem consistent with this finding. The high phthalate concentrations in freshly collected individuals suggest the presence in the environment of these compounds, and the ability of amphipods to assimilate them, while the decrease in phthalate concentrations in bioplastic-fed individuals could be attributed to the scavenging effect of virgin plastic, as already observed in a previous study. In summary, the results

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indicate that different bioplastics may have effects on *T. saltator* (i.e. survival rate and faecal pellets structure) and confirm a potential role of amphipods in the degradation of bioplastics in supralittoral zone of marine sandy beaches, even when bioplastics are not colonized by bacterial biofilm that seems to improve palatability.

## 1. Introduction

In recent years, the growing environmental awareness at business and societal level about the impact of conventional plastics, led to an increased demand of bioplastic, to replace non-biodegradable plastic materials of single use products, like shopping bags and packaging (Atiweh et al., 2021; Averous et al., 2001; Bastioli, 2001; Ezgi and Havva, 2015; Hottle et al., 2013; Melchor-Martínez et al., 2022; Wang et al., 2022). Bioplastics can derive from renewable resources (biobased), and can be biodegradable or non-biodegradable (Döhler et al., 2022; European Bioplastic, 2020). There are also exceptions represented by certain polymers derived from oil (fossil-based) but which are biodegradable (e.g. polybutyrate-adipate-terephthalate (PBAT) and polycaprolactone (PCL). (Albertsson et al., 2020; Goel et al., 2021). One of the EU directives outlines that by 2030, all plastic packaging placed on the EU market should be reusable or recycled cost-effectively (Calabrò and Grosso, 2018; European Bioplastic, 2020; European Commission, 2018; Venâncio et al., 2022). Efforts by companies to produce and advertise “green” plastics have received a very positive response from users, who associate the terms biodegradable, compostable or even just degradable with an environmentally friendly plastic that will decompose in a very short time without leaving a trace in the environment. However, the substitution of plastics with bioplastics poses many challenges and can lead citizens to mistakes and false perceptions, for example when it comes to the disposing of waste (Dilkes-Hoffman et al., 2019b, Dilkes-Hoffman et al., 2019a). In fact, the term biodegradability leads citizens to consider bioplastics as ‘environmentally friendly’ and therefore to underestimate the problem of their improper disposal. Biodegradability standards are valid under specific and controlled conditions of humidity, temperature, concentration and strain of microorganisms (Tong et al., 2022). On the other hand, the “fully compostable” packaging, that some companies are producing is identical to petroleum-based plastic, leading end-users to confuse normal plastic with compostable bioplastic and polluting recycling. Moreover, the fact that some biocompostable packaging cannot always be treated, when it ends up, in the organic waste collection.

Differences between laboratory conditions and the natural environment have raised doubts about the success of 100 % biodegradation of abandoned plastic waste in a short period of time.

There is still very limited research on the degradation of bioplastics in marine supralittoral environments of sandy shores; this lack of information is particularly concerning if we consider that marine environments are often severely the most affected by the deposition of various materials of natural or anthropogenic origin (Di Cesare et al., 2021; Dilkes-Hoffman et al., 2019b; Hodgson et al., 2018; Straub et al., 2017). Supralittoral zones of sandy beaches are characterized by high biodiversity, of which talitrid amphipods are typical representatives (Nakamura et al., 2022). Talitrid amphipods represent one of the largest components in terms of biomass in supralittoral zone and represent a major component of the food webs playing a key role in modification and fragmentation of organic matter of marine and terrestrial origin, representing a key element in the energy flow of stranded material (Dugan et al., 2003; Griffiths et al., 1983; McLachlan and Brown, 2006). Their detritivore and scavenging characteristics have led to the use of these crustaceans as a good bio-indicator of the human impact in the supralittoral zone of sandy shores and of environmental pollutants contamination (Rainbow et al., 1989; Rainbow and Phillips, 1993; Ugolini et al., 2012, Ugolini et al., 2008, Ugolini et al., 2005; Ungherese et al., 2012, Ungherese et al., 2010). Recent studies have evidenced that talitrid amphipods, such as *Cryptorchestia garbinii* and *Talitrus saltator*, can be one of the entry points for microplastics (MPs) in the trophic chain (Battistin et al., 2023; Iannilli et al., 2020, Iannilli et al.,

2018; Ugolini et al., 2013); moreover, MPs seem to play a double role as carriers and/or scavengers of contaminants in these crustaceans (Scopetani et al., 2018). After all, plastics has been found in all parts of coastal and marine ecosystem, and their presence in intertidal zone sediments would lead to interactions with a wide range of marine organisms (Accinelli et al., 2012; Venâncio et al., 2022). There is evidence that the ingestion of plastics items can result in harmful effects on the health of organisms (i.e. marine worms, larvae, bivalve corals, fishes, etc.), such as reduced growth rate and reproductive success (Browne et al., 2008; Tosetto et al., 2016; Uribe-Echeverría and Beiras, 2022; Wright et al., 2013). Recently, the growing bioplastic production is leading to an increased interest on the direct effects on the marine ecosystem and the aquatic organisms (Uribe-Echeverría and Beiras, 2022) but, to date, only few studies investigated bioplastic ingestion on amphipods (Hodgson et al., 2018; Shruti and Kutralam-Muniasamy, 2019; Straub et al., 2017). Due to natural (e.g. wind, tides, waves) or anthropic factors (e.g. touristic activities, fishing) a large part of the plastic material is deposited in marine-littoral environments (Geppetti and Tongiardi, 1967). The use of *T. saltator* as a bio-indicator of pollutants was tested for several contaminants (i.e. PAHs, PBDEs, trace metals, MPs) showing that these are mainly absorbed through food (Scopetani et al., 2018; Ugolini et al., 2012; Ungherese et al., 2012). However, little is known about the effects that the intake of bioplastics may have on these marine organisms. Therefore, the main objective of this study was to investigate the role of talitrid amphipods in the fragmentation and modification of bioplastics in absence of bacterial film, and the direct effects (i. e. survival rate, faecal pellets structure) of these materials on *T. saltator*. To this aim, a laboratory experiment was conducted under controlled conditions (photoperiod, temperature), feeding the individuals exclusively with two of the most common bioplastics that are likely to be found in the supralittoral zone. A multidisciplinary approach was applied, involving the application of different and innovative analytical techniques in this field, such as microtomography and NMR.

## 2. Material and methods

### 2.1. Amphipods collection

Adults *T. saltator* (length 7 - 10 mm, weight about 0.025 g each) were collected, one by one, on the beach of the Regional Natural Park of Migliarino, San Rossore, Massaciucoli (Pisa, Italy) in late spring-summer 2021 and 2022 by common entomology aspirators. The sandhoppers were brought to the laboratory and kept in Plexiglass boxes (0.25 L of capacity) with artificial damp sand; the experiment was conducted under controlled conditions ( $T = 25 \pm 2$  °C; artificial photoperiod L:D = 12:12 in phase with the natural ones). About 100 individuals were frozen immediately after sampling to evaluate the background content of phthalates and lipid.

Two different types of starch-based bioplastic commonly used in supermarkets carrier bags (named BIO1 and BIO2), classified as biodegradable and compostable according to UNI EN 13432, were chosen for the experiment. BIO1 and BIO2 were characterized by FTIR analysis in ATR mode with a IRAffinity-1S (SHIMADZU) equipped with the ATR sampling accessory (MIRacle™ PIKE Technologies) and GC-MS analysis (GC 7890A and MS 5975C Agilent Technologies) after extraction with methanol with methyl heptadecanoate as internal standard. Moreover, 3 mg of each bioplastic was completely dissolved in deuterated chloroform and analysed by  $^1\text{H}$  NMR on a Bruker Avance 400 MHz instrument for further characterization. Pools of about 20 *T. saltator* individuals were fed for 4 weeks with  $10 \times 10$  cm sheets of BIO1 or BIO2. Control experiments were conducted feeding pools of about 20 individuals with  $10 \times 10$  cm blotting paper

and dry fish food (SERA Vipran, Germany). From seven to eight replicas were carried out for each treatment. The amount of paper - dry fish food and bioplastic sheets was established on the basis of previous studies on the same species, preventing them from running out of food (Ciofini et al., 2020). Before the beginning of the experiment, individuals were fasted for 2 days. Bioplastics and paper with dry fish food samples were changed every week to prevent the growth of a bacterial film.

Survival rate was determined after 4 weeks, counting the individuals still alive. The consumption of BIO1, BIO2 and Control was monitored after one week by measuring the missing surface of the feeding sheets, taking digital photos of each. The images were processed by Photoshop (©Adobe) to reduce the number of colours in white (value = 255) and black (value = 0) tones, eliminating the halftones. Then, the percentage of the white surface compared to the black background was calculated for each sheet (e.g. see Fig. S1) by means of a MatLab (The MathWorks, Inc.) routine which, in the black and white matrix, counts the pixels of one colour respect to the total amount of pixels, obtaining its percentage.

## 2.2. Faecal pellets examination

Three faecal pellets of amphipods fed with BIO1 and BIO2 were characterized by FTIR analysis in ATR mode and the results were compared with those obtained by  $^1\text{H}$  NMR analysis following the above reported protocol.

Faecal pellets were also analysed by high-resolution 3D microtomography to evidence structural differences between BIO1 and BIO2 samples after transit through talitrid gut. The samples were analysed collecting  $\mu\text{m}$ -CT data using a Skyscan 1172 high resolution microCT at the University of Florence (Centro di Cristallografia Strutturale, CRIST). This system has a sealed, microfocus tungsten X-ray tube with a  $5\mu\text{m}$  focal spot size. The X-ray was produced by exposing the anode to an electron beam at a range of 72kV and 140  $\mu\text{A}$  to maximise the images contrast. Each sample was placed on a pedestal between the X-ray tube source and the CCD detector. The 2D X-ray images were captured with a slice-to-slice rotation angle range of 0.20.

## 2.3. Lipid content and phthalate esters determination

1 g of sandhoppers was homogenized with 2 g of  $\text{Na}_2\text{SO}_4$ , previously baked at  $400^\circ\text{C}$ , spiked with a surrogate standard (DEPH- $d^4$ ), to evaluate the method recovery and vortex extracted twice with a mixture of Dichloromethane and Hexane (3:1 v/v) followed by an ultrasonic bath. The extracts were combined, and 1 mL was used to the gravimetric determination of the lipid content; by solid-phase dispersive extraction (d-SPE) containing 1200 mg  $\text{MgSO}_4$ , 400 mg PSA, 400 mg C18 and 400 mg GCB (Bond Elute Dispersive Kit - Agilent Technologies). After centrifugation, the supernatant was collected and reduced in volume under a gentle stream of nitrogen. MEPH- $d^4$  was added as the internal recovery standard; subsequently, the extracts were analysed by GC-MS.

The presence of ten different phthalates (dimethyl phthalate (DMP), diethyl phthalate (DEP), diallyl phthalate (DAP), di-n-propyl phthalate (DPrP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBzP) dicyclohexyl phthalate (DCHP), bis(2-ethylhexyl) phthalate (DEPH), diisononil phthalate (DINP) and di-n-decyl phthalate (DNDP) were investigated.

The GC-MS analysis was performed by a Hewlett-Packard (HP) 6890 gas chromatograph equipped with an HP-5MS column (30 m  $\times$  0.25 mm  $\times$  0.25 mm), an HP 7683 autosampler, and an HP 5973 mass spectrometer following the operating condition reported elsewhere (Baini et al., 2017).

Quality control was performed by running procedural blanks together with the samples and subtracting the blank to contribute to the samples analysed. The mean recovery of the entire procedure was  $83.7 \pm 9.2\%$ , so data were not corrected for the recovery contribution.

## 2.4. Statistical analysis

Being the data on survival rate, consumption, and faecal pellet obtained from pools of individuals, the significance of the data was tested using non-

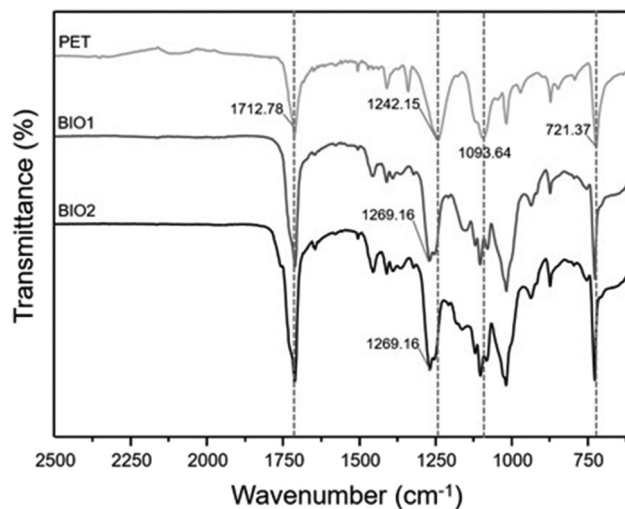


Fig. 1. Comparison between the ATR FTIR spectra of PET, BIO1 and BIO2. As shown, some diagnostic absorption bands of PET are displayed in the spectra of BIO1 and BIO2, although with small variations in frequencies for the absorption originally centred at  $1242\text{ cm}^{-1}$ , thus suggesting that the structural unit of PET is present in both the two bioplastics.

parametric tests. The G-test was used to compare the different experimental treatments with the survival rate. The Mann-Whitney  $U$  test (Siegel and Castellan, 1988; Zar, 1999) was used to compare the per capita consumption, lipid content and faecal pellet length of the different experimental and control groups. A significant difference was attributed when  $P \leq 0.05$ . Chemical data are presented as mean  $\pm$  Standard Deviation (SD).

## 3. Results and discussions

### 3.1. Bioplastics composition

The ATR analyses showed a similarity between the two plastics by tracing their spectra back to that typical of a polyester (PE). In particular, as shown in Fig. 1, the characteristic PET absorptions centred at 1712, 1242–1093 and  $721\text{ cm}^{-1}$  and respectively attributable to C=O stretching of the ester group, C—O stretching and out-of-plane bending of the aromatic protons (Jung et al., 2018), are also present in the spectra of BIO1 and BIO2. Analysis of the spectra shows that it is very likely that organic compounds such as terephthalic acid (TPA) and polyhydroxyalkanoates (PHA) are present in the formulation. The spectra may therefore be attributable to a PE, in particular very similar but not identical to PLA (polylactic acid), also showing the potential presence of longer chain fatty acids (e.g. succinic, adipic) in the formulation (Fig. 1).

Similarity but also few differences between the two plastic materials are also confirmed by GC-MS analysis, as reported in Table 1.

In particular, the presence of Levoglucosan, a sugar that represents a potential and additional nutrition source for individuals, has been evidenced only in BIO1 sheets, and it could explain the different mortality rate through the two experimental groups. In accordance with what assumed

Table 1  
Main composition of BIO1 and BIO2.

	BIO 1 (mg/kg)	BIO 2 (mg/kg)
Levoglucosan	$92 \pm 4.6$	–
1,6-Dioxacyclodecane-7,12-Dione	$120 \pm 14$	–
p-Dioxane- 2,5 dimethanol oligomers	–	$4300 \pm 280$
Butyl Phthalate	–	$92 \pm 33$
Eurecamide	$700 \pm 35$	$620 \pm 31$
TPA + 1,4-buthandiol + adipic acid oligomers	$1400 \pm 70$	$1300 \pm 65$

by ATR analysis, the presence of fatty acid chains was evidenced in both BIO1 and BIO2.

### 3.2. Survival rate

The survival rate recorded for BIO1 groups ( $n = 160$  87 %) were significantly higher than BIO2 ( $n = 130$ , 58 %) and Control groups ( $n = 120$ , 70 %) (BIO1 vs BIO2:  $G = 13.292$ ,  $df = 1$ ,  $p < 0.001$ ; BIO1 vs Control groups:  $G = 10.299$   $df = 1$ ,  $p < 0.01$ ; BIO2 vs Control groups:  $G = 0.053$ ,  $df = 1$ ,  $p = \text{NS}$ ). These results agree with reports on the composition of BIO1 and BIO2; in fact, a higher survival rate occurred in the experimental groups fed bioplastics with a potential nutrient source of sugar.

The estimated per-capita consumption of bioplastic and paper/dry fish food, calculated after 1 week dividing consumption by the number of individuals, did not show significant difference (BIO1 vs BIO2,  $U_{9,8} = 24$ ,  $p = 0.28$ ; BIO2 vs Control,  $U_{8,5} = 33$ ,  $p = 0.065$ ; BIO1 vs Control,  $U_{9,5} = 36$ ,  $p = 0.08$ ) although results showed a slightly preference for both bioplastics compared to Control.

The data obtained demonstrate the ability of *T. saltator*, a key element of the marine trophic chain, in consuming and ingesting bioplastics. This is consistent with what has been observed in other studies, where fragments of carrier bags have been used as food sources for amphipods. Hodgson and co-workers (Hodgson et al., 2018) highlighted the ability of *O. gammarellus* to ingest bioplastics fouled with microbial biofilm that is assumed to increase feeding/palatability. Straub et al. (2017) found that freshwater amphipods ingest bioplastics or conventional plastics equally

and no statistically significant differences in adverse effects on the amphipod *Gammarus fossarum*.

### 3.3. Faecal pellets examination

Faecal pellets of *T. saltator* fed with BIO1 and BIO2 were first examined by 3D microtomography to evidence any structural differences and compare to those from control groups. The first were characterized by clearly visible, short plastic filaments (Fig. 2a) while the second showed larger leaf-like structures within attributable to plastic material (Fig. 2b). The faecal pellets in the control experiments, being structurally characterized by a dense network, did not allow any structural analysis to be carried out (Fig. 2c).

The above microtomography data agree with ATR observations where significantly different absorption spectra are recorded for the experimental faecal pellet samples compared to the control. Comparison between BIO1 and BIO2 fragments length after gut transit showed significant differences ( $U_{20,20} = 30$ ,  $P < 0.002$ , Mann-Whitney  $U$  test). (Figs. 3A, S2, and S3).

Furthermore, overlaying the ATR spectra of BIO1 and BIO2 before and after sandhopper's intestinal transit, not valuable differences were evidenced between the native material and that excreted by the animal. However, small differences can be appreciated among the absorption spectra of BIO1 collected before and after gut transit, which can be attributed to the complex faecal matrix (see Figs. 3A and S2 of SI). Similar findings also emerged from the NMR analysis, that highlighted comparable spectra of faecal pellets when compared to the corresponding undigested items. As

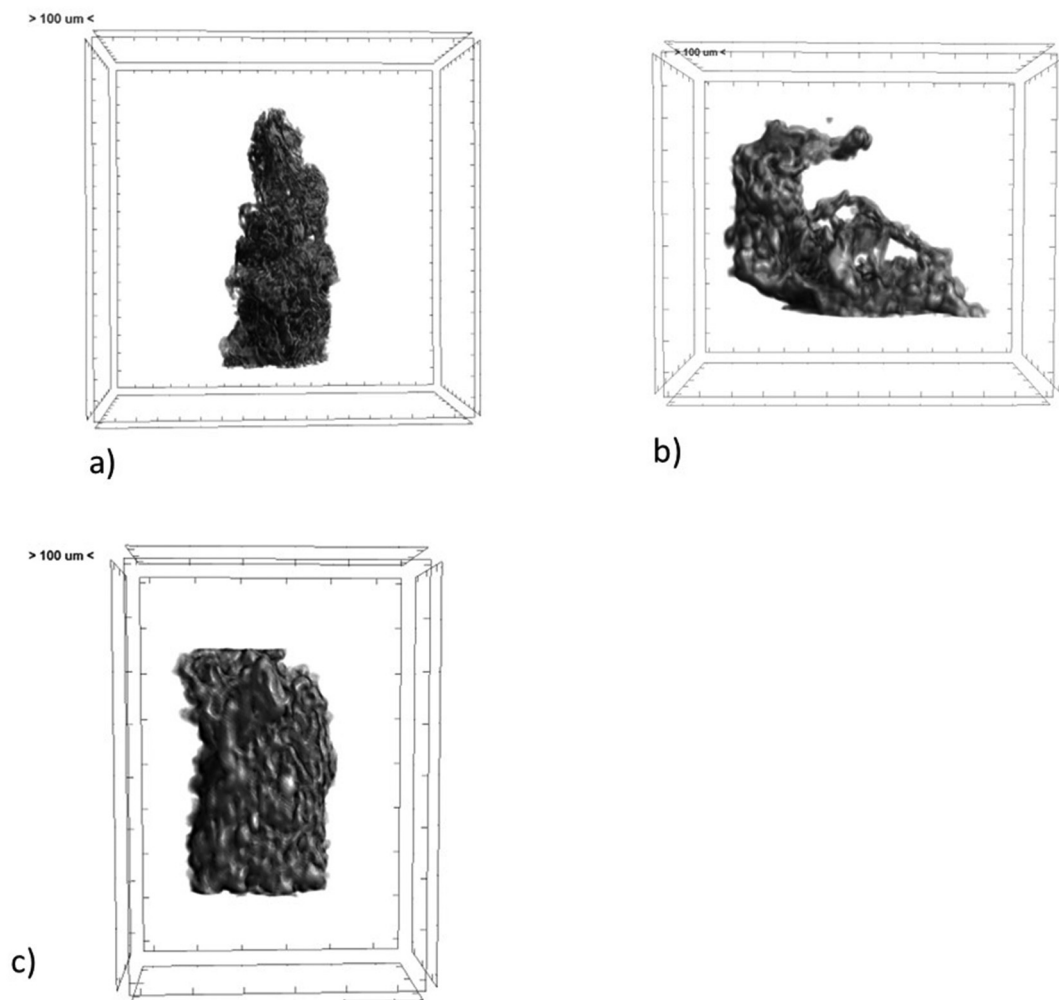


Fig. 2. 3D microtomography analysis of faecal pellets of *T. saltator* fed with BIO1 (a), BIO2 (b) and Control Experiment (c).

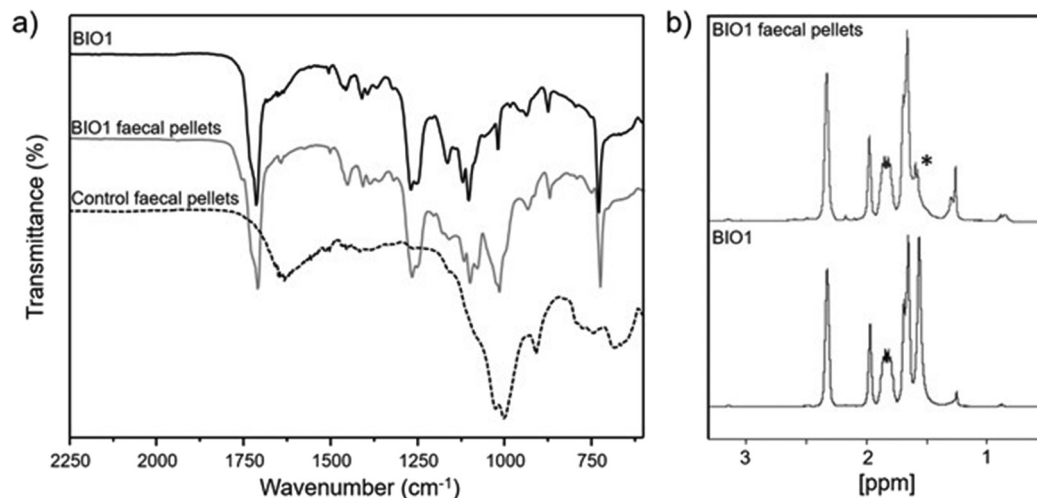


Fig. 3. ATR FTIR spectra of BIO1 before and after sandhopper's intestinal transit, along the one for the control faecal pellets (a) and NMR spectra of BIO1 before and after sandhopper's intestinal transit (3.2–0.5 ppm region, 400 MHz,  $\text{CDCl}_3$ ) (b).

shown in Figs. 3B and S3 of SI, these spectra featured a strong singlet signal in the aromatic region ( $\sim 8.10$  ppm), likely associated to equivalent hydrogen atoms gathered on the aromatic ring of PET, plus, a different set of signals within 4.43–4.08 and 2.32–1.30 ppm, attributable to protons belonging to the different aliphatic components of the polymer. Again, a slight but significant difference between BIO1 and the faecal pellets of individuals fed with it could be evidenced, as indicated, for example, by the decrease of the signal at 1.57 ppm in the spectrum of faecal pellets (Fig. 3B). No significant differences were instead observed in the case of BIO2. These findings may suggest the increased ability of *T. saltator* to consume the sugary and lipid components of BIO1 and could therefore provide an answer for the increased survival rates observed when amphipods are fed this polymer.

Summarizing, ATR and NMR analysis indicated slight differences in BIO1 composition before and after gut transit, suggesting a potential assimilation of sugars and lipids from bioplastics; this is possibly associated with the increased survival rate and higher lipid content of BIO1 experimental group. The microtomographic observations seem to indicate a greater difficulty in the fragmentation of BIO2 seem to be consistent with this hypothesis, as the faeces of the BIO2-fed talitrids, on the other hand, showed larger internal structures, suggesting less degradation in gut transit.

### 3.4. Lipid content evaluation and phthalates analysis

The data on lipid content are consistent with those observed for survival rate, i.e., a greater increase in percentages in the case of individuals fed BIO1 (see Table 2), even though the number of samples compared is low due to the low survival rate in BIO2. Lipid contents of *T. saltator* are significantly higher in BIO1 compared to BIO2 ( $U_{4,2} = 0, p = 0.028$ , Mann Whitney *U* test).

Phthalates were determined in the experimental and control groups as compounds related to plastic exposure. The data obtained were compared

**Table 2**  
Mean lipid content percentage (LC, %) and Phthalate esters concentration (ng/g lw).

		DIBP	BBZP	DEHP	DINP	$\Sigma_{10}$ Phthalates	LC
Natural individuals	Mean	22,214	667	34,767	2875	62,532	7.11
	Std. dev.	16,107	673	25,781	2900	39,476	2.26
Control	Mean	8343	323	16,061	363	25,137	3.98
	Std. dev.	11,872	516	22,728	430	35,236	2.92
BIO1	Mean	3133	25	1335	108	4747	1.7
	Std. dev.	1964	17	958	51	2105	0.4
BIO2	Mean	–	–	–	–	–	0.70
	Std. dev.	–	–	–	–	–	0.02

with those of freshly collected individuals (untreated samples) to verify natural background levels (Table 2). The presence of target phthalates was evident in all samples analysed, with a higher concentration in the untreated samples, followed by the control samples. A significantly lower concentration of total phthalates was determined in the BIO1 samples ( $U_{4,4} = 0, P = 0.028$ ), whereas a statistically significant analysis could not be made for samples from the BIO2 experimental group, due to the low survival rate.

Of the ten target phthalates, three compounds were found in all the samples: DEHP, DNIP and DIPB, representing the most common plasticising agents; BBZp was determined in 85 % of the samples analysed with lower concentrations than the other three (see Table 2). In all three groups, the DEHP was dominant confirming its large commercial use and ubiquities (Baini et al., 2017). Furthermore, it has been shown that the degradation of plastic material characterized by the presence of PET can lead to the release of DEHP, DIBP and BBZP (Keresztes et al., 2013; Pinto and Reali, 2009; Plotan et al., 2013), which are, also, the most abundant compounds found in the samples analysed. These results suggest that bioplastic could play the role of scavenger towards organic compounds, similarly with what has already been observed by Scopetani et al., 2018, regarding conventional plastic.

Thus, the potential release of phthalates from ingested bioplastics was also evaluated. The high phthalate concentrations in freshly collected individuals suggest the capacity of amphipods to assimilate such compounds in natural conditions. The decreased phthalate concentrations in BIO1-fed individuals compared to controls may be attributed to the scavenging effect of the 'virgin' plastic material, as already experimentally observed in a previous study (Scopetani et al., 2018), and/or to a scarce digestion and modification of the bioplastics used in this study.

## 4. Conclusions

This study proposes an innovative analytical approach to study and characterize microbioplastics in small sample amounts combining NMR, microtomography, ATR and GC–MS analysis. The potential role of supralittoral amphipods in the degradation of bioplastics in the environment is highlighted.

Data obtained show that *T. saltator*, like *O. gammarellus*, can ingest, fragment and partially modify biobased plastics used in the production of carrier bags. However, differences were found between experimental groups that consumed different bioplastics (BIO1 and BIO2) in terms of survival and fragmentation ability (and thus, probably, digestion). Furthermore, it should be noted that *T. saltator* inhabits sandy beaches with little washed-up organic material, unlike species of the genus *Orchestia*, such as *O. gammarellus*, that prefer seaweed and *Posidonia* banquettes. It is

known that *T. saltator* forages daily along the sea-land axis, heading towards the dune at night (Geppetti and Tongiardi, 1967). The upper part of the supralittoral does not favour the development of the bacterial film due to some important physical-chemical stress factors. Our results show that some bioplastics can be considered tasty even if they do not have microbial biofilms on their surface. This condition is the one that can be found in the supralittoral zone of sandy beaches. Therefore, given the role of *T. saltator* in the food chain of the supralittoral zone of sandy beaches (Griffiths et al., 1983; McLachlan and Brown, 2006), this species could provide a valuable contribution in the recycling/modification of starch-based bioplastics.

Finally, microbioplastic could play a dual role in pollutant transfer, acting as carrier (introducing pollutants into the body) but also as scavenger, removing those already in the gut. Not last, despite the same production and certification standards, the bioplastics used may have different impacts on *T. saltator*.

### CRedit authorship contribution statement

**Tania Martellini** – Conceptualization, Development of methodology, Investigation, Data Curation, Writing original manuscript, review and editing, supervision and project administration.

**Alessandro Russo** - Investigation, Data Curation, Writing original manuscript, review and editing.

**Alessandra Cincinelli** - Conceptualization, Data Curation, Writing, review and editing, supervision, resources and project administration.

**Saul Santini** - Development of methodology, Investigation, Data Curation, Writing, review and editing.

**Cristiana Lofrumento** - Investigation, Data curation.

**Matteo Baini** - Investigation, Data curation.

**Samuele Ciattini** - Investigation, Data curation.

**Luca Conti** - Investigation, Data Curation, Writing, review and editing.

**Francesca Mostardini** - Investigation, Data curation.

Luca Mercatelli-Software.

**Alberto Ugolini** - Conceptualization, Investigation, Data Curation, Writing original manuscript, review and editing, supervision, resources and project administration.

### Data availability

The authors are unable or have chosen not to specify which data has been used.

### Declaration of competing interest

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

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.162811>.

### References

Accinelli, C., Saccà, M.L., Mencarelli, M., Vicari, A., 2012. Deterioration of bioplastic carrier bags in the environment and assessment of a new recycling alternative. *Chemosphere* 89, 136–143. <https://doi.org/10.1016/j.chemosphere.2012.05.028>.

- Albertsson, A.C., Bødtker, G., Boldizar, A., Filatova, T., Prieto Jimenez, M.A., Loos, K., 2020. Biodegradability of Plastics in the Open Environment. Science Advice for Policy by European Academies, Evidence Review Report No. 8. <https://doi.org/10.26356/biodegradabilityplastics>.
- Atiwesh, G., Mikhail, A., Parrish, C.C., Banoub, J., Le, T.-A.T., 2021. Environmental impact of bioplastic use: a review. *Heliyon* 7, e07918. <https://doi.org/10.1016/j.heliyon.2021.e07918>.
- Averous, L., Fringant, C., Moro, L., 2001. Starch-based biodegradable materials suitable for thermoforming packaging. *Starch - Starke* 53, 368. [https://doi.org/10.1002/1521-379X\(200108\)53:8<368::AID-STAR368>3.0.CO;2-W](https://doi.org/10.1002/1521-379X(200108)53:8<368::AID-STAR368>3.0.CO;2-W).
- Baini, M., Martellini, T., Cincinelli, A., Campani, T., Minutoli, R., Panti, C., Finoa, M.G., Fossi, M.C., 2017. First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. *Anal. Methods* 9, 1512–1520. <https://doi.org/10.1039/C6AY02674E>.
- Bastioli, C., 2001. Global status of the production of biobased packaging materials. *Starch - Starke* 53, 351. [https://doi.org/10.1002/1521-379X\(200108\)53:8<351::AID-STAR351>3.0.CO;2-R](https://doi.org/10.1002/1521-379X(200108)53:8<351::AID-STAR351>3.0.CO;2-R).
- Battistin, G., Latella, L., Iannilli, V., 2023. Microplastic pollution in the food web: observation of ingestion by the talitrid amphipod *Cryptorchestia garbinii* on the shores of Lake Garda. *Eur. Zool. J.* 9, 73–82. <https://doi.org/10.1080/24750263.2022.2160019>.
- Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ. Sci. Technol.* 42, 5026–5031. <https://doi.org/10.1021/es800249a>.
- Calabrò, P.S., Grosso, M., 2018. Bioplastics and waste management. *Waste Manag.* 78, 800–801. <https://doi.org/10.1016/j.wasman.2018.06.054>.
- Ciofini, A., Yamahama, Y., Mercatelli, L., Hariyama, T., Ugolini, A., 2020. Specializations in the compound eye of *Talitrus saltator* (Crustacea, Amphipoda). *J. Comp. Physiol. A.* 206, 711–723. <https://doi.org/10.1007/s00359-020-01432-8>.
- Di Cesare, A., Pinnell, L.J., Brambilla, D., Elli, G., Sabatino, R., Sathicq, M.B., Corno, G., O'Donnell, C., Turner, J.W., 2021. Bioplastic accumulates antibiotic and metal resistance genes in coastal marine sediments. *Environ. Pollut.* 291, 118161. <https://doi.org/10.1016/j.envpol.2021.118161>.
- Dilkes-Hoffman, L., Ashworth, P., Laycock, B., Pratt, S., Lant, P., 2019a. Public attitudes towards bioplastics – knowledge, perception and end-of-life management. *Resour. Conserv. Recycl.* 151, 104479. <https://doi.org/10.1016/j.resconrec.2019.104479>.
- Dilkes-Hoffman, L., Lant, P.A., Laycock, B., Pratt, S., 2019b. The rate of biodegradation of PHA bioplastics in the marine environment: a meta-study. *Mar. Pollut. Bull.* 142, 15–24. <https://doi.org/10.1016/j.marpolbul.2019.03.020>.
- Döhler, N., Wellenreuther, C., Wolf, A., 2022. Market dynamics of biodegradable bio-based plastics: projections and linkages to European policies. *EFB Bioecon. J.* 2, 100028. <https://doi.org/10.1016/j.bioeco.2022.100028>.
- Dugan, J.E., Hubbard, D.M., McCrary, M.D., Pierson, M.O., 2003. The response of macrofauna communities and shorebirds to macrophyte wrack subsidies on exposed sandy beaches of southern California. *Estuar. Coast. Shelf Sci.* 58, 25–40. [https://doi.org/10.1016/S0272-7714\(03\)00045-3](https://doi.org/10.1016/S0272-7714(03)00045-3).
- European Bioplastic, 2020. *New Market Data 2019: Bioplastics Industry Shows Dynamic Growth*.
- European Commission, 2018. *PackagingWaste Directive (EU) 2018/852*. European Commission Brussels.
- Ezgi, B.A., Havva, D.O., 2015. A review: investigation of bioplastics. *J. Civ. Eng. Archit.* 9. <https://doi.org/10.17265/1934-7359/2015.02.007>.
- Geppetti, L., Tongiardi, P., 1967. Nocturnal migration of *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). *Monit. Zool. Ital.* 1, 37–40.
- Goel, V., Luthra, P., Kapur, G.S., Ramakumar, S.S.V., 2021. Biodegradable/bio-plastics: myths and realities. *J. Polym. Environ.* 29, 3079–3104. <https://doi.org/10.1007/s10924-021-02099-1>.
- Griffiths, C.L., Stenton-Dozey, J.M.E., Koop, K., 1983. Kelp wrack and the flow of energy through a Sandy Beach ecosystem. *Sandy Beaches as Ecosystems*. Springer, Netherlands, Dordrecht, pp. 547–556. [https://doi.org/10.1007/978-94-017-2938-3\\_42](https://doi.org/10.1007/978-94-017-2938-3_42).
- Hodgson, D.J., Bréchon, A.L., Thompson, R.C., 2018. Ingestion and fragmentation of plastic carrier bags by the amphipod *Orchestia gammarellus*: effects of plastic type and fouling load. *Mar. Pollut. Bull.* 127, 154–159. <https://doi.org/10.1016/j.marpolbul.2017.11.057>.
- Hottle, T.A., Bilec, M.M., Landis, A.E., 2013. Sustainability assessments of bio-based polymers. *Polym. Degrad. Stab.* 98, 1898–1907. <https://doi.org/10.1016/j.polymdegradstab.2013.06.016>.
- Iannilli, V., Di Gennaro, A., Lecce, F., Sighicelli, M., Falconieri, M., Pietrelli, L., Poeta, G., Battisti, C., 2018. Microplastics in *Talitrus saltator* (Crustacea, Amphipoda): new evidence of ingestion from natural contexts. *Environ. Sci. Pollut. Res.* 25, 28725–28729. <https://doi.org/10.1007/s11356-018-2932-z>.
- Iannilli, V., Corami, F., Grasso, P., Lecce, F., Buttinelli, M., Setini, A., 2020. Plastic abundance and seasonal variation on the shorelines of three volcanic lakes in Central Italy: can amphipods help detect contamination? *Environ. Sci. Pollut. Res.* 27, 14711–14722. <https://doi.org/10.1007/s11356-020-07954-7>.
- Jung, M.R., Horgren, F.D., Orski, S.V., Rodriguez, C.V., Beers, K.L., Balazs, G.H., Jones, T.T., Work, T.M., Brignac, K.C., Royer, S.-J., Hyrenbach, K.D., Jensen, B.A., Lynch, J.M., 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Mar. Pollut. Bull.* 127, 704–716. <https://doi.org/10.1016/j.marpolbul.2017.12.061>.
- Keresztes, S., Tatár, E., Czégény, Z., Zárny, G., Mihucz, V.G., 2013. Study on the leaching of phthalates from polyethylene terephthalate bottles into mineral water. *Sci. Total Environ.* 458–460, 451–458. <https://doi.org/10.1016/j.scitotenv.2013.04.056>.
- McLachlan, A., Brown, A.C., 2006. *The Ecology of Sandy Shores*. Elsevier <https://doi.org/10.1016/B978-0-12-372569-1.X5000-9>.
- Melchor-Martínez, E.M., Macías-Garbutt, R., Alvarado-Ramírez, L., Aratújo, R.G., Sosa-Hernández, J.E., Ramírez-Gamboa, D., Parra-Arroyo, L., Alvarez, A.G., Monteverde, R.P.B., Cazares, K.A.S., Reyes-Mayer, A., Yáñez Lino, M., Iqbal, H.M.N., Parra-Saldivar, R., 2022. Towards a circular economy of plastics: an evaluation of the systematic transition to a new generation of bioplastics. *Polymers (Basel)* 14, 1203. <https://doi.org/10.3390/polym14061203>.

- Nakamura, S., Yumioka, J., Kachi, S., Baba, Y., Kawai, S., 2022. Bacterial and fungal gut microbiota of supralittoral talitrid amphipods feeding on brown macroalgae and paper. *PLoS One* 17, e0279834. <https://doi.org/10.1371/journal.pone.0279834>.
- Pinto, B., Reali, D., 2009. Screening of estrogen-like activity of mineral water stored in PET bottles. *Int. J. Hyg. Environ. Health* 212, 228–232. <https://doi.org/10.1016/j.ijheh.2008.06.004>.
- Plotan, M., Frizzell, C., Robinson, V., Elliott, C.T., Connolly, L., 2013. Endocrine disruptor activity in bottled mineral and flavoured water. *Food Chem.* 136, 1590–1596. <https://doi.org/10.1016/j.foodchem.2012.01.115>.
- Rainbow, P.S., Phillips, D.J.H., 1993. Cosmopolitan biomonitors of trace metals. *Mar. Pollut. Bull.* 26, 593–601. [https://doi.org/10.1016/0025-326X\(93\)90497-8](https://doi.org/10.1016/0025-326X(93)90497-8).
- Rainbow, P.S., Moore, P.G., Watson, D., 1989. Talitrid amphipods (Crustacea) as biomonitors for copper and zinc. *Estuar. Coast. Shelf Sci.* 28, 567–582. [https://doi.org/10.1016/0272-7714\(89\)90047-4](https://doi.org/10.1016/0272-7714(89)90047-4).
- Scopetani, C., Cincinelli, A., Martellini, T., Lombardini, E., Ciofini, A., Fortunati, A., Pasquali, V., Ciattini, S., Ugolini, A., 2018. Ingested microplastic as a two-way transporter for PBDEs in *Talitrus saltator*. *Environ. Res.* 167, 411–417. <https://doi.org/10.1016/j.envres.2018.07.030>.
- Shruti, V.C., Kutralam-Muniasamy, G., 2019. Bioplastics: missing link in the era of microplastics. *Sci. Total Environ.* 697, 134139. <https://doi.org/10.1016/j.scitotenv.2019.134139>.
- Siegel, S., Castellan, N.J., 1988. *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill.
- Straub, S., Hirsch, P.E., Burkhardt-Holm, P., 2017. Biodegradable and petroleum-based microplastics do not differ in their ingestion and excretion but in their biological effects in a freshwater invertebrate *gammarus fossarum*. *Int. J. Environ. Res. Public Health* 14, 774. <https://doi.org/10.3390/ijerph14070774>.
- Tong, H., Zhong, X., Duan, Z., Yi, X., Cheng, F., Xu, W., Yang, X., 2022. Micro- and nanoplastics released from biodegradable and conventional plastics during degradation: formation, aging factors, and toxicity. *Sci. Total Environ.* 833, 155275. <https://doi.org/10.1016/j.scitotenv.2022.155275>.
- Tosetto, L., Brown, C., Williamson, J.E., 2016. Microplastics on beaches: ingestion and behavioural consequences for beachhoppers. *Mar. Biol.* 163, 199. <https://doi.org/10.1007/s00227-016-2973-0>.
- Ugolini, A., Borghini, F., Focardi, S., Chelazzi, G., 2005. Heavy metals accumulation in two syntopic sandhopper species: *Talitrus saltator* (Montagu) and *Talorchestia ugolini* bellan santini and Ruffo. *Mar. Pollut. Bull.* 50, 1328–1334. <https://doi.org/10.1016/j.marpolbul.2005.04.041>.
- Ugolini, A., Ungherese, G., Somigli, S., Galanti, G., Baroni, D., Borghini, F., Cipriani, N., Nebbiai, M., Passaponti, M., Focardi, S., 2008. The amphipod *Talitrus saltator* as a bioindicator of human trampling on sandy beaches. *Mar. Environ. Res.* 65, 349–357. <https://doi.org/10.1016/j.marenvres.2007.12.002>.
- Ugolini, A., Perra, G., Focardi, S., Somigli, S., Martellini, T., Cincinelli, A., 2012. Sandhopper *Talitrus saltator* (Montagu) as a bioindicator of contamination by polycyclic aromatic hydrocarbons. *Bull. Environ. Contam. Toxicol.* 89, 1272–1276. <https://doi.org/10.1007/s00128-012-0830-5>.
- Ugolini, A., Ungherese, G., Ciofini, M., Lapucci, A., Camaiti, M., 2013. Microplastic debris in sandhoppers. *Estuar. Coast. Shelf Sci.* 129, 19–22. <https://doi.org/10.1016/j.ecss.2013.05.026>.
- Ungherese, G., Mengoni, A., Somigli, S., Baroni, D., Focardi, S., Ugolini, A., 2010. Relationship between heavy metals pollution and genetic diversity in Mediterranean populations of the sandhopper *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). *Environ. Pollut.* 158, 1638–1643. <https://doi.org/10.1016/j.envpol.2009.12.007>.
- Ungherese, G., Cincinelli, A., Martellini, T., Ugolini, A., 2012. PBDEs in the supralittoral environment: the sandhopper *Talitrus saltator* (Montagu) as biomonitor? *Chemosphere* 86, 223–227. <https://doi.org/10.1016/j.chemosphere.2011.09.029>.
- Uribe-Echeverría, T., Beiras, R., 2022. Acute toxicity of bioplastic leachates to *Paracentrotus lividus* sea urchin larvae. *Mar. Environ. Res.* 176, 105605. <https://doi.org/10.1016/j.marenvres.2022.105605>.
- Venâncio, C., Lopes, I., Oliveira, M., 2022. Bioplastics: known effects and potential consequences to marine and estuarine ecosystem services. *Chemosphere* 309, 136810. <https://doi.org/10.1016/j.chemosphere.2022.136810>.
- Wang, L., Peng, Y., Xu, Y., Zhang, J., Liu, C., Tang, X., Lu, Y., Sun, H., 2022. Earthworms' degradable bioplastic diet of polylactic acid: easy to break down and slow to excrete. *Environ. Sci. Technol.* 56, 5020–5028. <https://doi.org/10.1021/acs.est.1c08066>.
- Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. *Curr. Biol.* 23, R1031–R1033. <https://doi.org/10.1016/j.cub.2013.10.068>.
- Zar, J.H., 1999. *Biostatistical Analysis*.