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Ingestion of chitosan-starch blends: Effect on the survival of supralittoral amphipods

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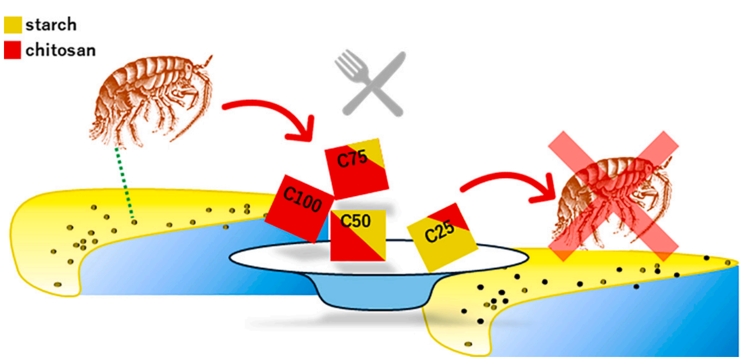
HIGHLIGHTS

- Biopolymers blends are likely to be released in the environment.
- Sandhoppers were fed with chitosan-starch mixtures at different percentages.
- Chitosan sheets were analysed before and after ingestion by ATR-FTIR.
- Sandhoppers fed with chitosan did not survive.

GRAPHICAL ABSTRACT



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ABSTRACT

Sandy beach ecosystems are particularly affected by plastic pollution. Supralittoral amphipods are important components of the food web in sandy beaches and their ability to ingest microplastics and bioplastics has been assessed. Chitosan, a polysaccharide obtained by deacetylation of chitin, the second most abundant polymer in the world, represents an interesting component to produce novel bioplastics in combination with other biopolymers like starch. Here, the possibility of ingesting chitosan-starch blends and the possible effects on the amphipod *Talitrus saltator* were investigated. Groups of adult individuals were fed with sheets containing mixtures of chitosan and starch in different percentages for 7 and 14 days. The results showed that chitosan ingestion is dependent on the percentage of starch present in the mixture. Moreover, FTIR analyses of both sheets and faecal pellets after consumption show that chitosan is not digested. Furthermore, the survival rate of amphipods fed with a mixture of chitosan and starch decreases after one week compared to the control groups (100 % starch and paper), and drops drastically to 0 % after two weeks the experiment began. In addition, consumption of 100 % chitosan is negligible. Therefore, the results of the experimental observations evidenced that chitosan is avoided as food resource and its consumption significantly affects the survival capacity of *T. saltator*. It is

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emphasized that the release of mixtures of chitosan and starch into the marine environment appears to be dangerous for littoral amphipods.

1. Introduction

Plastic pollution poses a threat to ecosystems worldwide (*e.g.* see Derraik, 2002; Browne et al., 2010, 2011; Andrady, 2011; Galgani et al., 2019), particularly sandy beach ecosystems (Scopetani et al., 2018; Horn et al., 2019; Serra-Gonçalves et al., 2019; Xu et al., 2020; Abelouah et al., 2022; Rose et al., 2024). The sandy shores are subject to accumulation of plant and animal material of marine and terrestrial origin. In this type of ecosystem, the stranded material is used by detritivorous, scavengers, and herbivorous organisms and degraded by bacteria (Griffiths and Stenton-Dozey, 1981; Griffiths et al., 1983; Porri et al., 2011; Nakamura et al., 2022). Among the beached materials, plastic and microplastic are often present in considerable quantities (Thompson et al., 2004; Kaberi et al., 2013; Lots et al., 2017; Martellini et al., 2018) and are ingested by many invertebrates (*e.g.* see Xu et al., 2020; Costa et al., 2023).

The amount of plastic waste entering the ocean is still uncertain, but studies (*i.e.* Jambeck et al., 2015; Zhu, 2021) estimated it at 4–23 million tonnes per year and is predicted to increase in the next years. Moreover, Baghi et al. (2022) reported that plastic production has nearly doubled in the last decade and is foreseen to rise further reaching 33 billion tonnes by 2050. Due to an increased awareness of the environmental impacts from conventional plastics, bioplastics are increasingly being proposed as a good environmentally friendly alternative (Averous et al., 2001; Bastioli, 2001; Hottle et al., 2013; Arikan and Ozsoy, 2015; Atiwesh et al., 2021; Melchor-Martínez et al., 2022).

One of the EU directives requires all plastic packaging placed on the market should be reusable or economically recycled by 2030 (Calabrò and Grosso, 2018; European Commission, 2018; European Bioplastic, 2020; Venâncio et al., 2022). As a result, many companies have produced and promoted “green” plastics, which have been positively received by consumers by associating bioplastic, biodegradable, compostable, or degradable with fast decomposition properties without environmental traces (Goel et al., 2021). The production of bioplastic has increased in recent years, reaching according to European Bioplastics, approximately 2.1 million tonnes in 2019, with a predicted growth to 2.4 million tonnes by 2024 (European Bioplastics, 2021; Baghi et al., 2022). Not all bioplastics are biodegradable (Albertsson et al., 2020; European Bioplastic, 2022) in open environment and, even if they are, the biodegradability standards are only valid under specific and controlled conditions of humidity, temperature, and microorganism concentration (*e.g.* Tong et al., 2022); thus, their improper disposal should not be underestimated. Therefore, the replacement of plastics by bioplastics may lead citizens to misbehave about waste disposal (Dilkes-Hoffman et al., 2019a, 2019b). Recently, the growing production of bioplastics and their abandonment in the marine and coastal environments has led to increased interest in the assessment of effects on aquatic organisms (see Teuten et al., 2009 for a review). Currently, chitosan might be a material of great interest for the bioplastic industry, being one of the most abundant linear polysaccharides on our planet. It is a partially *N*-deacetylated derivative of chitin, a biopolymer second only to cellulose in terms of abundance in nature. Chitosan is used for various industrial applications and as a biodegradable antimicrobial food packaging material competing with traditional non-biodegradable plastic-based materials. It is used in various sectors, such as biomedical, agriculture and water treatment. It is also used as a component in the cosmetic, textile, electronic, paper and food industries, and in some disposable products (for reviews see Yilmaz Atay, 2019; Zhang et al., 2021; Roman-Doval et al., 2023, Costa et al., 2024). Chitosan may have a lower environmental impact than traditional petroleum-based plastics as it is biodegradable and compostable (Leceta et al., 2013), however, even in this case, plasticizers and cross-linkers are used in some chitosan-containing products to improve their properties (Priyadarshi and Rhim, 2020). Furthermore, chitosan and its derivatives have attracted particular attention regarding their antimicrobial and antifungal activity (Kendra and Hadwiger, 1984; Muzzarelli et al., 1990; Tsai and Su, 1999; Li et al., 2002; Qi et al., 2004; Kong et al., 2010; Yilmaz Atay and Çelik, 2017; Aktuganov et al., 2018; Kidibule et al., 2021; Guarnieri et al., 2022).

Thus, chitosan appears to be a potential future replacement for many of the bioplastics currently in use. However, the negative effects of ingesting macro and microplastics on many marine organisms are well documented by an extensive literature on this topic (*e.g.* see Cole et al., 2011 and Guzzetti et al., 2018 for reviews) but despite the fact that marine sandy shores are among the main places of deposition of various types of plastics, only a few studies have investigated the effects of bioplastic ingestion by supralittoral arthropods (Tosetto et al., 2016; Hodgson et al., 2018; Carrasco et al., 2019; Shruti and Kutralam-Muniasamy, 2019; Martellini et al., 2023). Among these, it is known that many amphipod species are of considerable importance in the degradation of beached material and constitute a food resource for many invertebrates and birds, including migratory species. Furthermore, talitrid amphipods being herbivores, scavengers and grazers (Palluault, 1954; Lopez et al., 1977; Adin and Riera, 2003; Colombini et al., 2003, 2015; Olabarria et al., 2009; Porri et al., 2011; Abdelrhman et al., 2017) can absorb various types of pollutants; in fact, supralittoral amphipods, like *Talitrus. saltator* (Montagu) are good indicators of trace metals (Rainbow et al., 1999; Rainbow, 2002; Marsden and Rainbow, 2004; Ungherese and Ugolini, 2009; Ungherese et al., 2010a,b; Ugolini et al., 2004, 2005; Ugolini and Ungherese, 2012; Rochman et al., 2013; Jelassi et al., 2021), polybrominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs) (Ugolini and Ungherese, 2012; Ugolini et al., 2012; Ungherese et al., 2016) and it is demonstrated that they can ingest microplastics mixed with food (Ugolini et al., 2013; Hodgson et al., 2018; Scopetani et al., 2018; Iannilli et al., 2018; Carrasco et al., 2019).

The important role that amphipods play in the supralittoral zone of sandy coasts in the trophic chain of the detritus, consisting of the fragmentation and modification of stranded organic material, is well known (Palluault, 1954; Griffiths et al., 1983). Furthermore, amphipods constitute an important source of nutrition for various species of riparian birds, including migratory ones (Dauvin, 2024).

It seemed useful to consider the potential use of chitosan-starch blends, as components to produce new biocomposites, as a food source and the possible effects of their ingestion on the survival rate of supralittoral amphipods. The role of this species in marine coastal ecology as a bio-indicator for various organic and inorganic contaminants has been widely described, while, to the best of our knowledge, the effects that the intake of bioplastics of different compositions may have on it remain rather unexplored. With the aim of filling this gap, this study explores the effect, under controlled conditions (temperature, photoperiod), of chitosan/starch blends in different percentages and in the absence of a bacterial film on the survival of *T. saltator*.

2. Materials and methods

Adult specimens of *T. saltator,* as it is one of the most common species of the supralittoral of sandy coasts present from the Mediterranean to the European coasts of the Atlantic Ocean, North Sea and Baltic Sea, were used for the experiments. A work-flow of the experimental procedure is reported in Supplementary Information (Fig. S1). The animals (about 200 individuals) were collected one by one using entomological aspirators in the spring-summer of 2022 and 2023 in the Migliarino San Rossore Massaciuccoli Regional Natural Park (Pisa, Fiume Morto Vecchio locality, 43°44′55" N; 10°16′31″ E). The amphipods were transported in boxes with wet sand to the laboratory in Florence within 3 h from the collection and placed in plastic tanks (17 × 11 × 13 cm). Each tank was divided in half by a 3 cm high plastic partition glued to the bottom so that the artificial sand (Amtra deko) in one half could be moistened with seawater, while the other remained dry. The temperature was regulated at 25 ± 2 °C, the artificial lighting cycle (L:D = 12:12) was in phase with the natural one.

In each tank, 10 individuals fasted for 2 days were placed before the start of the experiment. A total of 30 or 40 individuals were tested for each experimental treatment. All the food sources used were made up of 5 × 5 cm sheets of 1) absorbent paper and dry food for aquarium fish (Sera Vipan, Germany), standard food normally used to keep sandhoppers in captivity even for a long time (controls); 2) 100 % starch; 3) 100 %-chitosan; 4) 75 %-chitosan mixed with 25 %-starch; 5) 50 %-chitosan mixed with 50 %-starch; 6) 25 %-chitosan mixed with 75 %-starch. No additives, like plasticizers, were added to the samples.

Chitosan mixed with starch sheets were developed using the solvent casting method (Gupta et al., 2022). 100 %-Starch sheets were prepared dissolving 400 mg of corn starch (Sigma Aldrich) in 50 ml of hot distillated water at 80 °C, with continuous stirring for 15 min. The resulting solution was then poured into a Petri dish (10 × 10 cm) and dried. Once the solvent evaporated, a 100 % starch sheet was formed. For 100 %-chitosan sheets, 400 mg of chitosan (Sigma Aldrich – low molecular weight) was solubilized in 50 ml of 2 % acetic acid at 60 °C, stirred magnetically for 10 min, and then poured into a Petri dish to dry. To prepare the 75 %-chitosan – 25 %-starch sheets, the polymers were solubilized separately. Firstly, 100 mg of starch powder was dissolved in 15 ml of hot distillated water at 80 °C under magnetic stirrer for 15 min. At the same time, 300 mg of chitosan was solubilized in 35 ml of 2 % acetic acid at 60 °C with stirring for 10 min. Subsequently, the starch solution was added to chitosan solution, thoroughly mixed to obtain a homogeneous solution, and poured into a Petri dish until it is dried. Similar procedures were followed for 50 %-chitosan – 50 %-starch sheet and 25 %-chitosan – 75 %-starch sheet, with changes in amount of powder and solvent. For the preparation of the first sheet, two solutions were prepared with 200 mg of powder dissolved in 25 ml of solvent, while for the second one, 100 mg of chitosan in 15 ml of acetic acid and 300 mg of starch in 35 ml of water were used (Li et al., 2013). The resulting sheets had a thickness of 0.02 mm.

For each treatment 7 days after the introduction of the sheets into the tanks (one sheet of one type per tank placed on the dry sand), the surface not eaten by the amphipods was measured in relation to the total sheet surface. This was possible thanks to a specially written software in the MatLab environment (The MathWork, Inc.) capable of detecting the white (uneaten surface) and black (eaten surface) pixels that compose the photographic image in the absence of halftones, of each sheet at the end of the experiment (Fig. 1, see also Martellini et al., 2023). Furthermore, for each treatment, the number of individuals still alive 7 and 14 days after the start of the experiment was considered.

2.1. Chemical analyses

To investigate the possible degradation of chitosan following intestinal passage, feces from sandhoppers exposed to 50 %-chitosan-50 %-starch and 100 %-starch were collected within two days of deposition. As a control, analyses were also conducted on 50 %-chitosan – 50 %-starch, 100 %-starch, and 100 %-chitosan samples taken from unused sheets. These analyses were carried-out by an IRAffinity-1S by SHIMADZU equipped with the ATR sampling accessory (MIRacleTM PIKE Technologies) and GC–MS analysis (GC 7890 A and MS 5975C Agilent Technologies), in the 4000–500 cm^{-1} range. Additionally, the faecal pellets obtained from the amphipods were subjected to FTIR analysis.

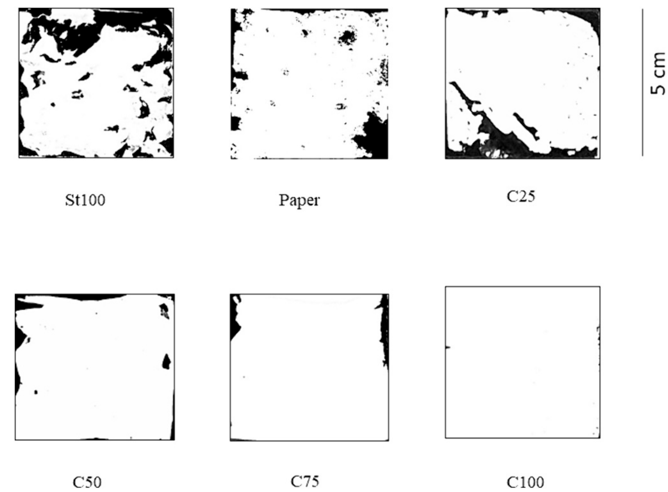

Fig. 1. Photographs of the consumption of polymers by *T. saltator* after 7 days. Only the most representative images, chosen *ad hoc*, of all the photos of the sheets used are shown. The uneaten surface is the white one. 100 %-starch (St100), paper, 25 %-chitosan mixed with 75 %-starch (C25), 50 %-chitosan mixed with 50 %-starc- (C50), 75 %-chitosan mixed with 25 %-starch (C75), and 100 %-chitosan (C100).

2.2. Statistical analysis

Statistical analyses were performed using SPSS (IBM). Comparisons between the different treatments relating to consumption were carried out using the Mann-Whitney *U* test while for comparisons between treatments relating to survival the G test was used (Siegel and Castellan Jr., 1988; Zar, 1999). The limit of statistical significance was set at $P \leq 0.05$.

3. Results

3.1. Consumption

After 7 days in the tanks (Fig. 2) it was evident that the 100 %-chitosan sheet was not eaten by the sandhoppers as the sheet appears practically intact. The non-consumed chitosan area was significantly larger than that found for all other treatments ($P = 0.032$, Mann Whitney U test, it was the worst value obtained among all comparisons). Among the comparisons between the uneaten parts relating to sheets containing chitosan and starch in different percentages, the only statistically significant one was between 75 %-chitosan and 25 %-chitosan, the latter being preferred by sandhoppers ($P = 0.021$, Mann Whitney U test). Therefore, 25 %, 50 %, and 75 %-chitosan seemed more palatable than the polymer with the highest percentage of chitosan. The comparison between paper and 100 %-starch does not reach statistical significance ($U = 18$, $P = 0.365$) whereas all comparisons between sheets containing chitosan in various percentages *vs* 100 %-starch and *vs* paper are significant ($U = 7$, $P = 0.05$ in the worst case: 50 %-chitosan *vs* paper).

3.2. Survival rate

A high survival rate is observable after 7 days (Fig. 3A) both in animals fed with paper (26/30, 90 %) and in those fed with 100 %-starch (27/30, 87 %) (Fig. 3, $G = 0.151$, $df = 1$, P=NS, G test). A high survival rate (21/30, 70 %) was also recorded in the presence of 100 %-chitosan, in fact the comparisons between survival with 100 %-chitosan *vs* 100 %-starch and *vs* paper were not fully statistically significant, $G = 3.728$, $df = 1$, $0.05 < P < 0.1$, G test, in the best case) while the comparisons between 100 %-chitosan *vs* 75 %-chitosan ($G = 5.210$, $df = 1$, $P < 0.05$, G test) and reached significance chitosan −100 % *vs* 50 %-chitosan (G =

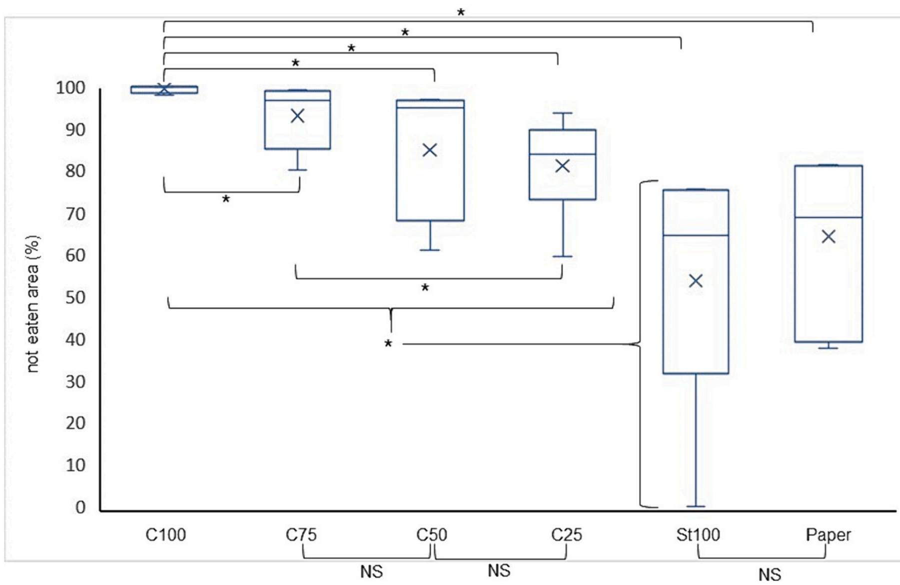

Fig. 2. Box-plot diagrams of the uneaten area after 7 days of the sheets of paper, 100 %-starch (St100), paper, 25 %-chitosan mixed with 75 %-starch (C25), 50 %-chitosan mixed with 50 %-starch (C50), 75 %-chitosan mixed with 25 %-starch (C75), and 100 %-chitosan (C100). The asteriscs indicate the probability level ($P < 0.05$) of the comparisons among groups (Mann Whitney *U* test). See text for further information and more details about statistical analysis (Section 3.1)

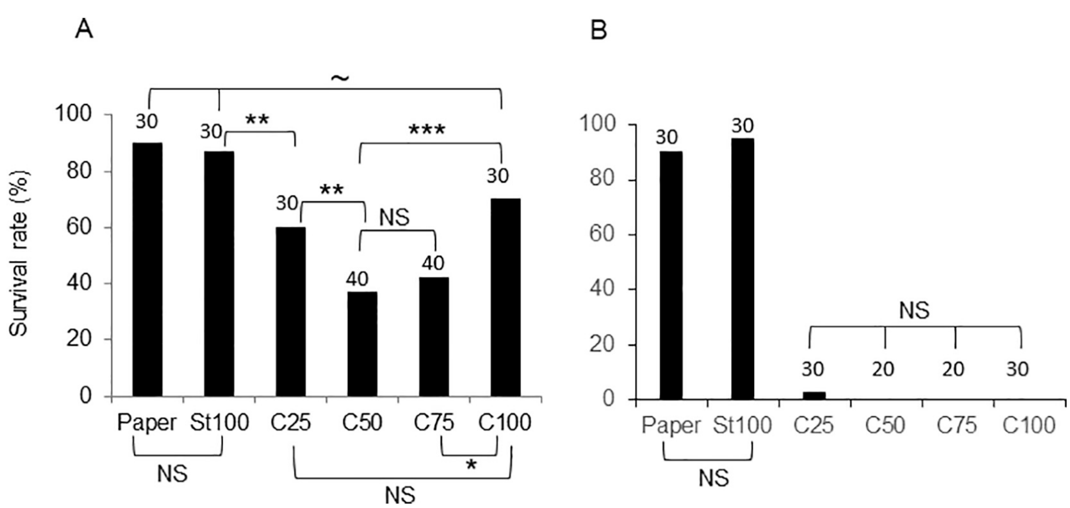

Fig. 3. Survival rate of *T. saltator* after 7 and 14 days (A and B, respectively) in the presence of paper sheets, 100 %-starch (St100), paper, 25 %-chitosan mixed with 75 %-starch- (C25), 50 %-chitosan mixed with 50 %-starch (C50), 75 %-chitosan mixed with 25 %-starch (C75), and 100 %-chitosan (C100). The numbers above the bars indicate the number of tested individuals. The asterisks indicate the probability level of the comparisons among groups (G test) (* = P < 0.05, ** = $P < 0.02$, *** = $P < 0.01$, ~ means $0.05 < P < 0.1$). See text for further information and more details about statistical analysis (see Section 3.2).

7.247, df = 1, $P < 0.01$, G test). The comparison between 100 %-chitosan *vs* 25 %-chitosan was not statistically significant (G = 0.643, df = 1, P=NS, G test) The comparisons between paper or 100 %-starch and the number of survivors exposed to sheets containing different percentages of chitosan and starch were all statistically significant (the worst level was related to the comparison between 100 %-starch *vs 25 %*-chitosan, G = 5.461, df = 1, $0.01 < P < 0.02$).

After 14 days (Fig. 3B), it could be noted that the survival in the presence of 100 %-chitosan was 0 %. The same was observed for 75 %-chitosan and 50 %-chitosan. Even for 25 %-chitosan the survival was low (3 %). In the comparison among the survivals recorded in the presence of the sheets with the different percentages of chitosan, full statistical significance was never reached; the best level was achieved in the comparison between 100 %-chitosan *vs* 25 %-chitosan (G = 3.699, df = 1, 0.05 < P < 0.1, G test). Even the comparison between paper and 100 %-starch did not reach statistical significance (G = 0.314, df = 1, P=NS, G test) and a high survival of *T. saltator* was evident (paper = 90 %, starch = 95 %). Comparisons between 7 and 14 days showed no significant differences in the number of sandhoppers surviving in the presence of paper (G = 0.231, df = 1, P=NS, G test) or 100 %-starch (G = 0.910, df = 1, P=NS, G test). However, comparisons made between the number of survivors after 7 and 14 days within each treatment with sheets containing different percentages of chitosan were all significant: the 7 *vs* 14 days comparison with 25 %-chitosan was the worst result (G = 17.318, df = 1, $P < 0.001$, G test).

3.3. Chemical analyses

In Fig. 4A are reported the ATR-FTIR spectra for 30 %-chitosan compared to 100 %-chitosan and 100 %-starch, which show the typical absorption bands of these two polysaccarides (Hebeish et al., 2009; Drabczyk et al., 2020). Although, the spectra of the two polysaccharides

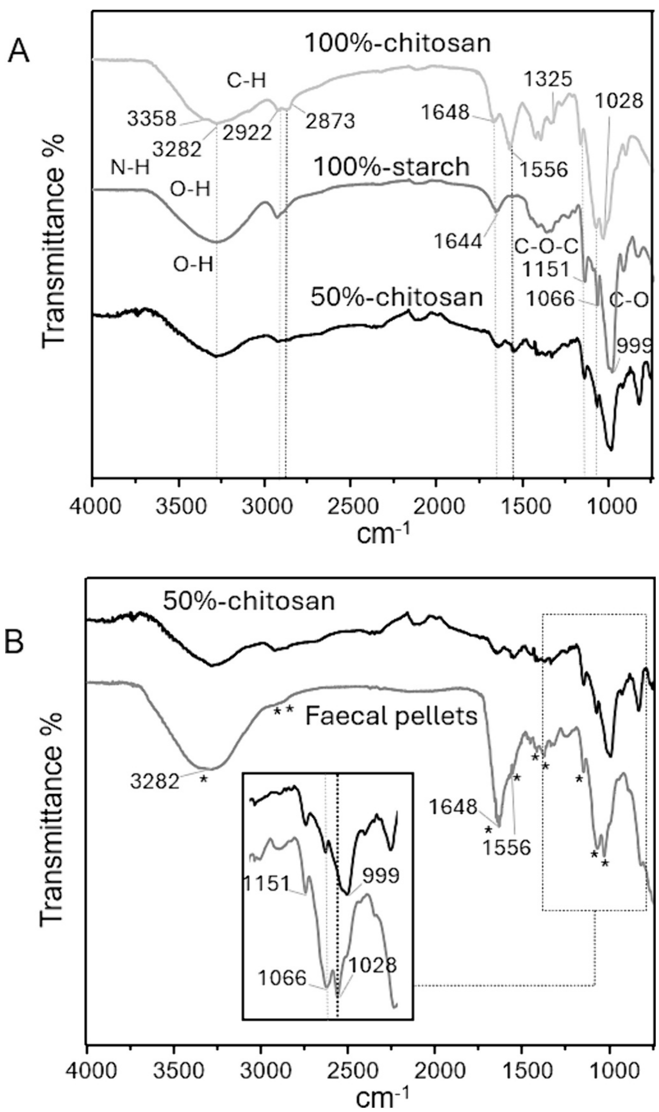

Fig. 4. A, ATR-FTIR spectra of 100 %-chitosan, 100 %-starch, and 50 %-chitosan-with the main absorption bands; B, comparison between spectra obtained for chitosan-50 % and its corresponding faecal pellets. The stars indicate the diagnostic absorptions of starch while in the insets is shown a focus on the 1450–990 cm^{-1} region highlighting the main differences between the spectra of chitosan −50 % (feeding sheet) and faecal pellets.

100 % could seem very similar, significant differences can be evidenced in their FTIR profiles. Observing the adsorption region between 1151 and 999 cm^{-1}, associated to the asymmetric stretching of the C-O-C bridge and the C—O stretching (Wang and Xie, 2010), can be evidenced that the C—O stretching of starch-100 % is significantly shifted towards lower frequencies, of *c.ca* 29 cm^{-1}, if compared to that of 100 %-chitosan. This is of particular interest in the comparative analysis between the FTIR spectra of 50 %-chitosan and faecal pellets (see Fig. 4B, inset).

As expected, the FTIR spectrum of 50 %-chitosan (light grey, Fig. 4A) contains all the signals separately displayed by its components, thus confirming the presence of both polysaccharides in the mixed sample.

The comparison between the FTIR spectra of 50 %-chitosan and of its corresponding faecal pellets is reported in Fig. 4B. As shown, in the profile of faecal pellets can be still appreciated the absorption bands of both chitosan and starch, thus hinting at their simultaneous presence in the sample. Interesting information is then achieved by analysis of the representative fingerprint region of spectra. More, in this region the spectrum of faecal pellets exhibits the typical triplet of absorptions of chitosan, as it can be easily appreciated from the inset of Fig. 4B, where it is visible the last C—O stretching resonating at 1028 cm^{-1}, instead of at 999 cm^{-1} as in 50 %-chitosan. This can be assumed as diagnostic of a predominant contribution of chitosan in the infrared spectrum of faecal pellets indicating a probable digestion of the starch component of the polymeric matrix. Outside this region, the differences found at 1648 and 1558 cm^{-1} among the two spectra are less reliable to gain information regarding the composition of faecal pellets as, on one side, vibrations of chitosan in this region have been generally reported to significantly vary depending on the particular chitosan fabrication (Kwon and Jeong, 2020), and, importantly, the absorption of starch at ~1642 cm^{-1} is strongly affected by the highly variable crystallinity of commonly commercialized starches (Kizil et al., 2002).

Therefore, despite a quantitative estimation cannot be made based on the sole IR data, taken together these data suggest a preferential digestion of the starch-component of the polymer, in line with the predominant presence of the chitosan component in the faecal pellets witnessed by infrared analysis.

4. Discussion

The main achievement of our research is that the ingestion of chitosan significantly affects the survival capacity of *T. saltator* and seems that chitosan is avoided as a food resource.

The high survival rates after both 7 and 14 days in the starch-100 % and paper (*i.e.* cellulose) treatments show that these elements may constitute an important trophic resource for supralittoral amphipods. These findings align with current knowledge on the feeding habits of supralittoral talitrids, although some species-specific differences are known (Mengoni et al., 2013; Abdelrhman et al., 2017). In fact, *T. saltator*, grazer and scavenger feeds on stranded detritus of both animal and vegetable origin. In the categories with different percentages of chitosan the survival rates to the seventh day vary greatly. With chitosan-25 % there is a survival rate of 60 %, with chitosan-50 % and 75 % the survival drops to 37 % and 42 %, respectively but, surprisingly, in the presence of chitosan-100 % survival increases (70 %). However, after 14 days, while with paper or 100 % starch survival remains practically unchanged compared to 7 days, survival in the presence of chitosan drops to 0 % regardless of the percentage contained in the food sheet (Fig. 3).

Despite the reduced surface area consumed (Fig. 2), the survival rate after 7 days in 100 %-chitosan appears so high could be due to the use of temporary food sources, such as feces or dead individuals. However, these very limited food sources do not seem sufficient to cover nutritional needs for 14 days.

In accordance with the above, the most consumed sheets were paper and 100 %-starch. Among the sheets containing chitosan the highest consumption was observed for 25 %-chitosan; this is probably due to the higher percentage of starch making it more palatable, while for 100 %-chitosan the consumption was almost absent. It should be also noted that sandhoppers fed with 100 %-starch and paper survive in greater numbers than individuals fed with sheets with various percentages of chitosan for which higher mortality rates are found. This was further evidenced by the FTIR analysis of faecal pellets from individuals fed with different feeding sheets (Fig. 4). Furthermore, the difference in survival could also be attributed to alterations in the digestive system due to chitosan ingestion. In fact, following ingestion, chitosan tends to swell, creating a physical bulk that can prevent the correct processing of food within the digestive system and inducing a false perception of satiety; moreover, chitosan is also known for reducing dietary fat absorption in the intestine (Cheung et al., 2015). It has also been demonstrated that in *Tubifex tubifex* worms chitosan induced oxidative stress (Mosleh et al., 2007). Furthermore, chitosan possesses antimicrobial properties, being effective against the growth of some bacteria and fungi (Kendra and Hadwiger, 1984; Muzzarelli et al., 1990; Roller and Covill, 1999; Tsai and Su, 1999; Li et al., 2002; Qi et al., 2004; Carlson et al., 2008a, 2008b; Xing et al., 2015; Kidibule et al., 2021; Dou et al., 2024) and once ingested by *T. saltator* chitosan could likely influence its digestive capabilities by altering or making inefficient the intestinal microbiome (Mengoni et al., 2013; Abdelrhman et al., 2017).

5. Conclusions

The ingestion of chitosan mixed with starch significantly affects the survival capacity of *T. saltator* within 14 days from the ingestion. Therefore, these results, suggest that the use of high percentage of chitosan to produce novel biocomposites, can be more dangerous for littoral fauna than other single components to produce starch-based bioplastics, as recently demonstrated on the same species (see Scopetani et al., 2018; Martellini et al., 2023). Beyond the evidence that chitosan is not a nutronal source for *T. saltator* a possible detrimental effect, associated to the antibacterial properties of this polymeric component, can be also envisaged.

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Declaration of generative AI in scientific writing

No AI or AI-assisted tools were used during the preparation of our manuscript.

CRediT authorship contribution statement

Alberto Ugolini: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Alessandro Russo:** Writing – review & editing, Investigation, Data curation. **Jessica Costa:** Methodology, Investigation. **Alessandra Cincinelli:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition. **Tania Martellini:** Writing – review & editing, Methodology, Investigation, Funding acquisition. **Luca Conti:** Writing – original draft, Investigation, Funding acquisition. **Duccio Cavalieri:** Writing – review & editing, Visualization, Funding acquisition. **Luca Mercatelli:** Writing – original draft, Software, Methodology, Investigation. **Rebecca Pogni:** Writing – review & editing, Visualization, Validation, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicting interests that influenced this research.

Data availability

Data will be made available on request.

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