Note

# Litter in alien species of possible commercial interest: The blue crab (*Callinectes sapidus* Rathbun, 1896) as case study

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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

Marine litter levels were measured in the stomach contents, hepatopancreas, and gonad tissues of crustacea decapod (*Callinectes sapidus* Rathbun, 1896; $n = 6$), a widespread alien species affecting the Lesina lagoon. Results highlight a mean of 2.5 (SD = 1.6) items/animal and indicate the presence of metals fragments (13%) and plastics (13% PE; 6.7% PET) in the stomach contents of collected individuals. No microplastic particles were detected in the hepatopancreas or in muscle tissue, while microplastic fibres (nylon, rayon, polyester) were found present in female specimen gonads. The presence of synthetic fibres in the investigated species reflects the relative contamination level in this habitat type and suggests that the blue crab could be considered a model organism for evaluating the contamination status of the study area.

## 1. Short note

Alien species could pose a significant threat for both the conservation of marine ecosystems and preservation of marine industries such as fishing and tourism (Bax et al., 2003). Marine coastal areas are fragile ecosystems subject to high human pressure and can be characterized by having a low resilience. Therefore, the introduction of a new allochthonous species could lead to changes in (Mahoney and Bishop, 2017) the existing autochthonous community. Coastal lagoons represent naturally stressed ecosystems (Zaldívar et al., 2008) where fluctuations of environmental parameters are extensive and climate and trophic state, especially in terms of nutrients and phytoplanktonic biomass, are frequently changing (Kjerfve, 1994; Specchiulli et al., 2010). The green crab *Carcinus aestuarii* Nardo (1847) is an autochthonous species traditionally harvested in Mediterranean areas. Starting from the beginning of the 20th century, the alien species blue crab, *Callinectes sapidus* (Rathbun 1896), was introduced in Europe, coming from the western Atlantic Ocean, and immediately competed with the autochthonous species (Mancinelli et al., 2017b). Blue crabs are a key functional component of benthic food webs (Dittel et al., 2006) foraging on sediment. They are often characterized by omnivorous opportunistic deposit-feeding trophic habits consisting of plants, invertebrates, conspecifics, and carcasses (Dittel et al., 2006; Seitz et al., 2011). In addition, an adult can reach a size > 20 cm in length. Direct agonistic activity of individual blue crabs interferes with the foraging of others, making *Callinectes sapidus* species a highly competitive species (Clark et al., 1999). The wide distribution of *C. sapidus* recorded over the course of a few decades indicated that colonization together with its predator and belligerent behaviour made this species a possible threat for ecosystem conservation, therefore, it is among the 100 worst invasive alien species in the Mediterranean Sea (Streftaris and Zenetos, 2006). Nevertheless, this species is representative of an interesting protein resource (containing 14–19%) for human exploitation, due to its body size (Martínez et al., 2017) leading scientists to consider it as a key feeding resource with no threat to human health (Mancinelli et al., 2017a; Streftaris and Zenetos, 2006). Additionally, blue crab fishing could exemplify a useful management strategy in reducing numbers of individuals in alien species population to offer positive effects on local biodiversity (Mancinelli et al., 2017b). Costa et al. (2018) reported that crabs can use marine debris to construct burrows, with a preference for soft plastic (30%), straw, rope and foam (21%); furthermore, they can actively ingest any kind of particles, including litter, of a maximum dimension comparable with their buccal apparatus.

The present study aims to assess litter levels in blue crabs collected from a largely colonized Mediterranean lagoon (Cilenti et al., 2015)

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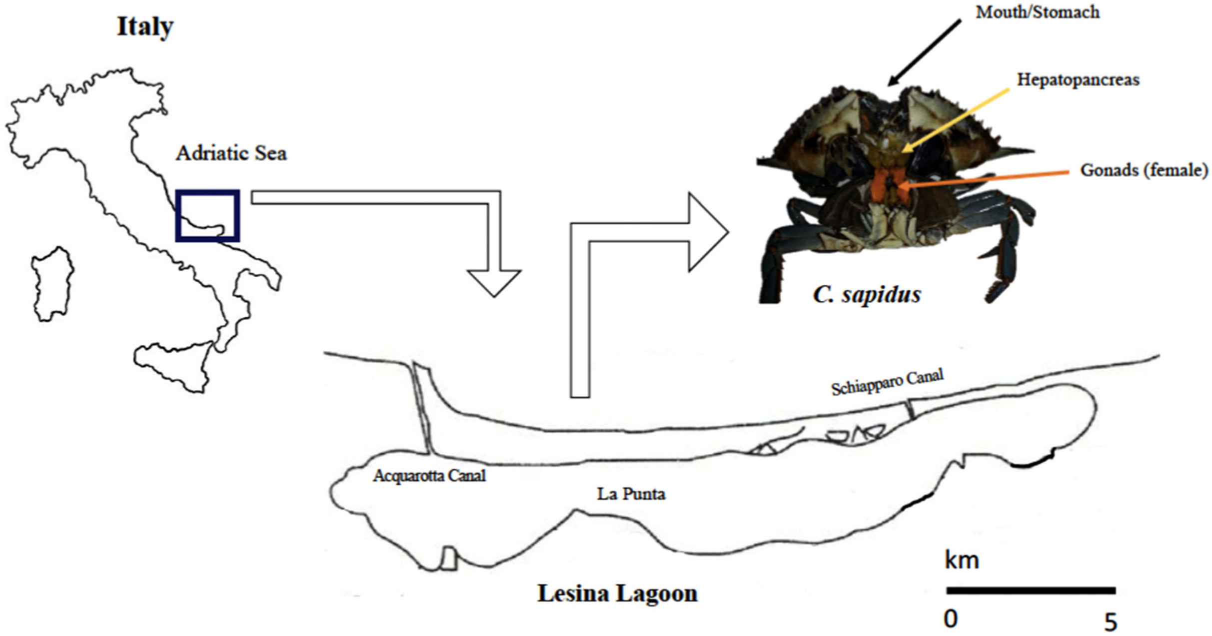


**Fig. 1.** Study area and sampled species. The geographical localization of Lesina Lagoon is reported also showing a map highlighting the general ecosystem structure. Furthermore, a representation of animal's tissues selected for the analyses is included.

where the potential use of this species as a feeding resource could represent an interesting local economic opportunity (Mancinelli et al., 2017a). The analysis of litter in their stomach content is a key step in evaluating: i) bottoms levels of pollution; ii) possible threats to human health as a feeding resource; iii) the utility of using blue crab as a species for the biomonitoring of litter impacts in coastal areas. In this study, different tissues (stomach, muscle, hepatopancreas, and gonad) were analyzed separately to assess the risks related to translocation of the smallest particles (< 150 μm) in different organs (Lusher et al., 2017).

The Lesina lagoon (Apulia, Italy) was selected as the study area of choice (Fig. 1) because it is often affected by eutrophication events, which have already resulted in a severe dystrophic crisis during 2008 (Cilenti et al., 2010; Specchiulli et al., 2010; Basset et al., 2013). It is a largely exploited lagoon ecosystem by humans, where fishing and aquaculture activities represent a significant source for the local economy (Breber et al., 2009). Blue crab organisms ($n = 6$) were collected using nets by local fisherman, transported to a laboratory setting and immediately sacrificed by exposure to −4 °C for 2 h. Biometrics such as carapace length (CL; 0.1 mm), width (CW; 0.1 mm), weight (WW; 0.1 g), and sex determinations were performed and related values (mean, SD; min-max) were recorded (Table 1). Animals were dissected on a precleaned working surface, paying attention to minimizing the exposure of the animals' tissues to air as much as possible in an attempt to avoid microplastic contamination. Critical activities were performed under a fume hood equipped with HEPA filters to reduce cross-contaminations of samples by airborne pollution. Collected tissues were then stored in precleaned glass bottles and weighed (w.w.), the whole organ was analyzed per animal.

**Table 1**
Biometrical features of collected blue crabs. Data are reported as mean, standard deviation (SD) and range of variability(min-max). Weight of each analyzed tissues were reported. Percentage of sex were 33.3% males versus 66.7% female. Carapace length (CL; LOD = 0.1 mm), width (CW; LOD = 0.1 mm), wet weight (WW; LOD = 0.1 g). Hp, hepatopancreas; G, gonads; S, stomach; M, muscle. Maximum values are associate to males while minimum to females' specimens.

| | CW | CL | WW | WWHp | WWG | WWS | WWM |
|---|---|---|---|---|---|---|---|
| | mm | Mm | g | g | g | g | g |
| Mean | 169.2 | 75.2 | 311.1 | 13.7 | 4.2 | 3.9 | 2.9 |
| SD | 21.8 | 11.8 | 193.3 | 3.1 | 2.3 | 1.9 | 0.9 |
| Min | 146.0 | 63.0 | 150.3 | 10.7 | 2.3 | 1.3 | 2.2 |
| Max | 195.0 | 90.0 | 558.1 | 17.6 | 7.1 | 6.1 | 4.0 |

Microplastic extractions were performed with Creon digestion (37 °C; TRIS-buffered pH). Creon digestion was selected because it has been shown to be a highly efficient digestion technique allowing for the quick removal of animal tissue without risking the ability to correctly identify plastic polymers with μFT IR (von Friesen et al., 2019).

Digested tissues were filtered using a filtering apparatus fitted with a paper fibre filter disk (pore diameters 0.6 μm); air exposure was minimized to reduce potential airborne pollution. Filters were stored in glass Petri dishes and dyed with Rose-Bengal (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein, Sigma-Aldrich) with respect to literature recommendations to help highlight desired particles or content on the filter (Ziajahromi et al., 2017). Rose-Bengal stains non-plastics particles such as natural fibres (cotton, cellulose) with a pink colour allowing the scientist to quickly overlook them while searching for particles intended for chemical analyses, accelerating the sorting process and reducing false positives.

The petri dishes were then dried overnight at 40 °C. A stereomicroscopic presorting was performed at 10–80× (SMZ-800 N); targeted items were measured by a digital webcam (DS Fi3) managed by the software NIS-elements D (Nikon®). Collected targets were then analyzed via microscopy associated with Fourier Transform Infrared Spectroscopy (μFT-IR; NicoletiN10 MX, ThermoFischer Scientific®Waltham, MA, USA) equipped with aMCT-A (spectral range: 7800–650 $cm^{-1}$) detector cooled with liquid nitrogen to determine their chemical composition. Particles thinner than 35 μm were analyzed using the reflection mode, collecting spectra on several points per particle. Identifications were performed by determining the percentage of spectral match of targeted items with respect to referenced spectral libraries of normal and aged microplastics (OMNIC™ Picta™ software libraries) integrated with original laboratory spectral libraries developed by years of related research (threshold for the spectra back-recognition was > 75% of match; LOD = 10 μm of size). Experimental blanks (negative controls) were analyzed to evaluate any false-positive

**Table 2**
Marine litter in stomach contents of *C. sapidus*. In Table 2a, data are reported as mean, standard deviation (SD) and range of variability (min-max) and expressed as number of items per specimens (#). In Table 2b, results related to microparticles size (μm) are summarized. Data are reported as total means and grouped according to microparticles shape and colours. NC = not calculable. (*) for very thick particles, width × length is recorded (1487 × 6,191 μm). Recoveries of microplastics in blanks (< 0.2 items/L recorded for any microplastic types). Concerning frequencies: about tissues, 83.3% of animals' stomach contents contain micro-litter while only female specimen showed microfibres in gonads (33.3%; n = 6). As regards as litter shapes, 66.7% of the micro-litter recorded in stomach contents are fibres; while 50% are fragments. Spherules and films are not recorded. In females' gonads 100% of recorded micro-litter are fibres.

| Table 2a | | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| #Items | | 2.5 | 1.6 | 0.0 | 4.0 |
| #Fibres | Black | 0.7 | 0.5 | 0.0 | 1.0 |
| #Fibres | Blue | 1.2 | 1.2 | 0.0 | 3.0 |
| #Fragment | Light brown | 0.2 | 0.4 | 0.0 | 1.0 |
| #Fragment | Red | 0.2 | 0.4 | 0.0 | 1.0 |
| #Fragment | Black | 1.7 | 3.6 | 0.0 | 9.0 |

| Table 2b | | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| Size fibres | Black | 564.4 | 378.9 | 146.3 | 913.9 |
| Size fibres | Blue | 1592.8 | 702.6 | 580.3 | 2106.2 |
| Size fragment | Light brown | (*) | NC | – | 6191.0 |
| Size fragment | Red | 102.5 | NC | 102.5 | 102.5 |
| Size fragment | Black | 1448.5 | NC | 1448.5 | 1448.5 |

occurrences due to indoor plastic pollution from the laboratory. Deionized pre-filtered (0.45 μm; $n$ = 5; 100 mL) water samples were treated as a tissue sample and evaluated to quantify indoor pollution. Data reported on samples were not corrected according to the recoveries in blanks.

Collected animals were adults in their reproductive stage; body size (maximum carapace dimension) ranged from 15 cm (smallest female) to 20 cm (largest male). Microparticles were recorded in 50% of the analyzed stomach content. Frequencies of the presence/absence of microparticle were sex independent (i.e. both males and females showed 50% of occurrence). The maximum value of recorded microparticles was 4.0 per animal (female). The number of animals per sex did not allow for results to be tested on a statistical basis; evident differences in stomach content levels were not reported comparing sex. The mean number of confirmed items per specimens (SD; min-max range), grouped by particle shape and colour, are reported in Table 2. Table 2 also shows the mean size of recorded microparticle items (SD; min-max). Ingested particles showed dimensions higher than 100 μm, with only an occasional record of microparticles higher than 150 μm. Very large particles were ingested by blue crabs larger than 1.5 × 6.1 mm (female). Studies show that particles smaller than 150 μm could be translocated from the stomach towards other tissues (Lusher et al., 2017). Nevertheless, the hepatopancreas and muscle tissues appeared to be unpolluted by microparticles in all the tested animals as well as the gonads in males. On the contrary, gonads of the female specimens showed the presence of synthetic fibres. In relation to the chemical composition of recorded litter (Fig. 2) it is worth noting that plastics and metal-made particles were also recorded. Coloured synthetic fibres represented > 60% of microparticles found in the blue crab tissues. With regard to the non-synthetic fibres recorded; it is interesting to note that the largest part is represented by coloured cellulose fibres that could be derived from cotton-made human clothes.

Coastal lagoons are ecosystems particularly affected by litter pollution, a relatively new type of pollutant found in aquatic ecosystems and of international concern (Barnes et al., 2009). In 2017, plastic production increased to 348 million tonnes, representing the main component of litter in aquatic environments (Plastics Europe, 2018). A

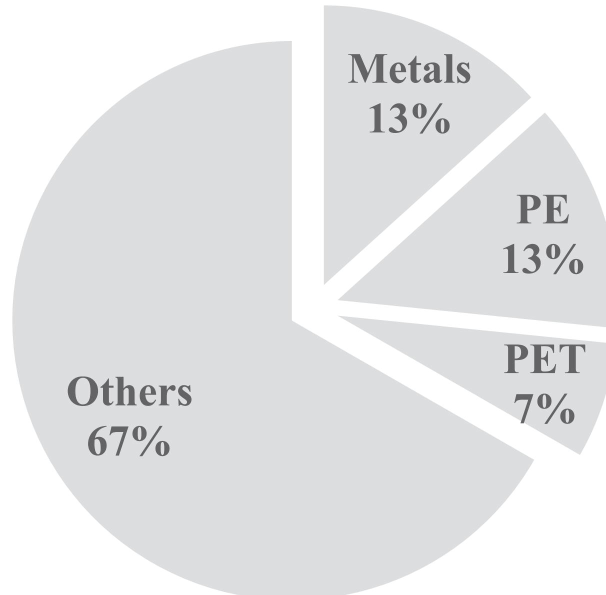


**Fig. 2.** Chemical compositions (%) of litter recorded in blue crabs. Data are expressed as percentage of recorded chemical type over the total amount recorded. Mean results (n = 6). Others includes nylon, rayon, polyester, cellophane.

particular concern related to possible ecological and ecotoxicological consequences by plastic pollution in aquatic environments results from microplastics and their interactions with the biotic compartment (Wright et al., 2013; Gall and Thompson, 2015). Particles accumulated in sediments could become available, through ingestion, for many benthic species (Thompson et al., 2004). Moreover, an interesting recent study has provided evidence that a benthic crustacean could contribute to the presence of fragment microplastics through its scavenging activity and digestion (Cau et al., 2020). According to the findings in the existing literature, the stomach of blue crabs could act as a size-bottleneck for ingested microplastics, enhancing the retention of larger particles within the stomach and promoting fragmentation into smaller plastic debris, which is then released in the intestine. Recently, the relationship among different ecosystem types and microplastic levels recorded in the stomach contents of benthic species has been reported on holothurians (Renzi et al., 2018), highlighting that microplastics could be able to penetrate marine trophic web (Ivar Do Sul and Costa, 2014; Setala et al., 2014). Furthermore, another study highlighted that benthic species prefer fibres (Neves et al., 2015). Frequencies of the occurrence of microplastics lower than 50% in commercial and abundant species are reported by most studies (Digka et al., 2018 and references therein), while higher percentages were found in sardines from some Mediterranean areas (Avio et al., 2015; Renzi et al., 2019). Micro-litter occurrence in the stomach contents of analyzed animals in this study include large plastic and metal particles at frequencies of 50% of all tested animals indicating that the described results could be used for assessing the contamination status of other study systems. Recent literature highlights that marine invertebrates can ingest microparticles and plastic made particles with sizes comparable to their feeding resource, i.e. plankton (Browne et al., 2008). Our results show that blue crabs actively ingest large fragments of a wide variety of materials. It has been demonstrated that there is a selective predation behaviour with respect to some colours and shapes of microplastics (i.e. black and blue filaments and fragments) has been reported for fishes (Renzi et al., 2019) and holothurians (Graham and Thompson, 2009). The latter indicated a preference for plastic fragments due to selecting this shape with tentacles within other plastic shapes during in vitro studies (Graham and Thompson, 2009). Similar studies could be of particular interest to improve the scientific communities understanding of the feeding behaviour of blue crab towards micro-litter and to define this species as an acceptable biomonitoring species for marine litter in coastal lagoons. The presence of synthetic fibres observed in this study in female gonads could reflect habitat type and the contamination level of the habitat, the latter representing important determinants in microplastics exposure and accumulation as well as the possibility for females to accumulate microfibres by direct contact of mature gonads on sediment. Microfibres recorded in marine environments largely originate from the household washing of synthetic fabrics (Corami et al., 2019). These resulting floating microfibres could be trapped by the gonads in mature females. Furthermore, our results show that blue crab edible tissue (muscle) could be considered safe for human consumption. Nevertheless, the ingestion of large plastic and metal pieces by this species could expose sea animals to bioaccumulation of metals and other organic chemicals potentially released from the digestion process of ingested microparticles. For this reason, further studies will be performed to better clarify the assumptions made here.

## CRediT authorship contribution statement

**Monia Renzi:** Conceptualization, Project administration, Supervision, Writing - original draft, Writing - review & editing. **Lucrezia Cilenti:** Funding acquisition, Investigation. **Tommaso Scirocco:** Investigation. **Eleonora Grazioli:** Formal analysis. **Andrea Broccoli:** Investigation. **Valentina Pauna:** Investigation. **Francesca Provenza:** Validation, Writing - original draft. **Antonietta Specchiulli:** Conceptualization, Writing - review & editing, Funding acquisition.

## 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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