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Evidence of uses of marine litter by Mediterranean Cephalopoda

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Understanding the interaction between marine biota and marine litter is essential to comprehend how organisms face the continuous alteration of their habitats due to anthropogenic pressure. The evidence here reported, based on both direct *in situ* observations and citizen science obtained information, refer to the interactions between two Mediterranean Cephalopoda species and marine litter in natural environments, and, more specifically, between: i) *Octopus vulgaris* and a plastic bin used for laying eggs inside, ii) *Sepia officinalis* and an artificial Egi lure, used for oviposition, iii) another specimen of *O. vulgaris* and an artificial lure, used for den enhancing. This highlights marine litter presence, probably joint with the scarcity of natural substrates, could lead to a behavioural adaptation of the species in responding to the reproductive stimulus, especially in the case of semelparous species. Based on these individual observations it is undoubtedly of crucial importance improving the knowledge base on the relationship between marine biota - marine litter and effects on marine animals and offspring.

KEYWORDS

marine litter, Cephalopoda, recreational fisheries, interactions, behavioral responses

1 Introduction

The widespread presence of marine litter in the marine environment poses a serious threat to the health of marine ecosystems and biota (Gall and Thompson, 2015; Laskar and Kumar, 2019). One of the main contributions, in terms of marine litter, is ascribable to both professional and recreational fishing activities. This kind of pollution principally derives from the abandoned, lost and/or discarded fishing gear (ALDFG) and recreational fishing marine debris (RFMD) (Gilman et al., 2021; Watson et al., 2022). In recent decades, this global phenomenon has aroused the interest of the scientific community and various types of interaction between marine fauna and marine litter have been documented by numerous researchers worldwide. Main types of interaction reported include ingestion, entangling, and colonizing, with special regard to marine litter, mainly plastic one (Macali et al., 2018; Savoca et al., 2019a, b, 2020; Woods et al., 2019; Amaral-Zettler et al., 2020; Capillo et al., 2020; Bruno et al., 2022; D'Iglio et al., 2022; Du et al., 2022; Mancuso et al., 2022). In addition, many behavioural studies have focused on the above-mentioned subjects, with particular attention to cephalopods (Freitas et al., 2022, 2023). Cephalopoda are known to be the most intelligent invertebrates with complex behavioural intra and inter specific interactions: benthic cephalopods can use objects (both of natural and artificial origin) to carry out activities during their whole life span (O'Brien et al., 2021; Hoffmann, 2022). Several examples have been reported, from sheltering to reproduction, including intraspecific communication (Amodio et al., 2019; Chung et al., 2022). In this regard, the citizen science approach is an effective tool for gathering information on behavioural interactions between cephalopods and artificial objects in the marine environment. A comprehensive example is the study conducted by Freitas et al. (2022), in which the authors identified 8 genera and 24 species of benthic octopuses interacting with marine litter through the collection of 261 underwater images from citizen science records. Of all documented types of interaction, the most frequent was sheltering (n = 178; 68.2%), followed by being on top of the litter (n = 30; 11.5%), burrowing (n = 26; 10.0%), moving out of litter (n = 18; 6.9%), and stilt-walking (n = 9; 3.5%). The interactions recorded in Freitas et al. (2022) were with hard objects of glass (41.6%), followed by plastic (24.7%), metal (17.6%), organic (8.6%), and others (organic, miscellaneous, undetermined, rubber, cloth) with lower percentages.

Regarding the sheltering of octopuses, it is known that their complex life cycle and reproductive behaviours are related to the selected shelter characteristics, such as small opening, sufficient internal space to host the specimen, and availability of stones, seashells and other objects around the opening (Katsanevakis and Verriopoulos, 2004a; Guerra et al., 2014).

Benthic cephalopods use hard substrates as a shelter to hide and defend themselves from predators or to disappear from their prey, helped by the camouflage. The use of hard substrates by cephalopods also occurs during the reproductive phases (Freitas et al., 2022, 2023): octopuses and cuttlefish mainly use hard substrates for attaching their fertilized eggs (Guerra et al., 2016; Pedà et al., 2022).

Female octopuses deposit, protect, and ventilate their eggs in shelters, and the choice of the latter affects the reproductive success of the species (Iribarne, 1990; Katsanevakis and Verriopoulos, 2004a). A shelter's characteristics are of fundamental importance for the protection of both the individual from predators, particularly during daytime, and the developing eggs. The reproduction of the octopuses, and of course the reproductive efficiency, is strongly influenced by the availability of suitable shelters to be enhanced and used (Katsanevakis and Verriopoulos, 2004b). Regardless of the reproductive purpose, it is common for *Octopus vulgaris* (Cuvier, 1797) and other Octopoda species, to use the selected shelter as a den, where the specimens add stones, seashells and, in some cases, litter for enhancement purposes and for improving the efficacy of all den types (Katsanevakis and Verriopoulos, 2004a; Guerra et al., 2014).

Differently from octopuses, cuttlefish have a more free-living lifestyle during the non-reproductive periods, showing nektobenthic behaviour with occasional low to medium (Zatylny-Gaudin and Henry, 2018) amplitude migrations depending on various factors (Adamo et al., 2000; Guerra et al., 2016; O'Brien et al., 2017; Zatylny-Gaudin and Henry, 2018). In the case of the Mediterranean cuttlefish, *Sepia officinalis* (Linnaeus, 1758), the reproductive stimulus is what triggers migration. During the breeding season, specimens of *S. officinalis* move to shallow waters where they mate and reproduce (Bloor et al., 2013). Hereafter, the female selects a substrate to lay fertilized eggs in. Normally, the egg laying location, the oviposition site, is characterized by optimal ecological and environmental conditions for the survival and subsequent growth of the offspring (Pierce et al., 2008; Hastie et al., 2009).

Octopus vulgaris and *S. officinalis*, are semelparous spawners (Rodríguez-Rúa et al., 2005). These spawners are characterized by a single or at least a double (rarely) reproductive event, in which a single batch of eggs is deposited within their lifetime (Rocha et al., 2001). The deposition site selection is therefore of fundamental importance for the reproductive fitness of the species (Iribarne, 1990). Octopuses seek as safe a shelter as possible for egg laying, and normally use natural environments, like rocky substrates and/or building a den in muddy/sandy bottoms, both for protection, and, in the case of females, also for egg laying. In terms of protection, site selection is crucial for these species, which have lost their shell during evolution (Packard, 1972; Aronson, 1991), and the absence of shelter in some substrates represents a limiting resource for the distribution of the species (Katsanevakis and Verriopoulos, 2004b; Katsanevakis et al., 2007; Freitas et al., 2023).

How reproduction behaviours of these Cephalopoda, in terms of selected structures for egg laying, are influenced by the presence of marine litter is a topic worthy of interest but that still presents many knowledge gaps.

The aim of the present study is to report some evidence of *O. vulgaris* and *S. officinalis* interaction with marine litter during the final act of reproduction, the oviposition, and of den enhancing by *O. vulgaris*, highlighting the importance to better explore this kind of behaviours to understand how wild animals face environmental modifications due to marine litter pollution.

2 Method

2.1 Sampling area and sites description

The sampling area was the Strait of Messina and surrounding coastal areas (Figure 1).

The Strait of Messina (Sicily, Italy) is well-known to marine biologists for its peculiar and diversified marine habitats, inhabited by a very rich faunal assemblage (De Domenico et al., 1976; D'Iglio et al., 2023). This area divides the Tyrrhenian Sea (northern part) from the Ionian Sea (southern part) including important, protected habitats such as the long-shore rocky structure known as Messina Beachrock and the Capo Peloro lagoon with its two basins (Ganzirri and Faro marshes). Strait of Messina area, due to its proximity to the city of Messina, is characterized by anthropic influences such as domestic pollution and small-scale fisheries, mainly practiced by recreational fishers. The two Seas surrounding the Strait of Messina suffer anthropic pressure too. The entire area of the southern Tyrrhenian Sea is subject to massive anthropic pressure, related to the presence of

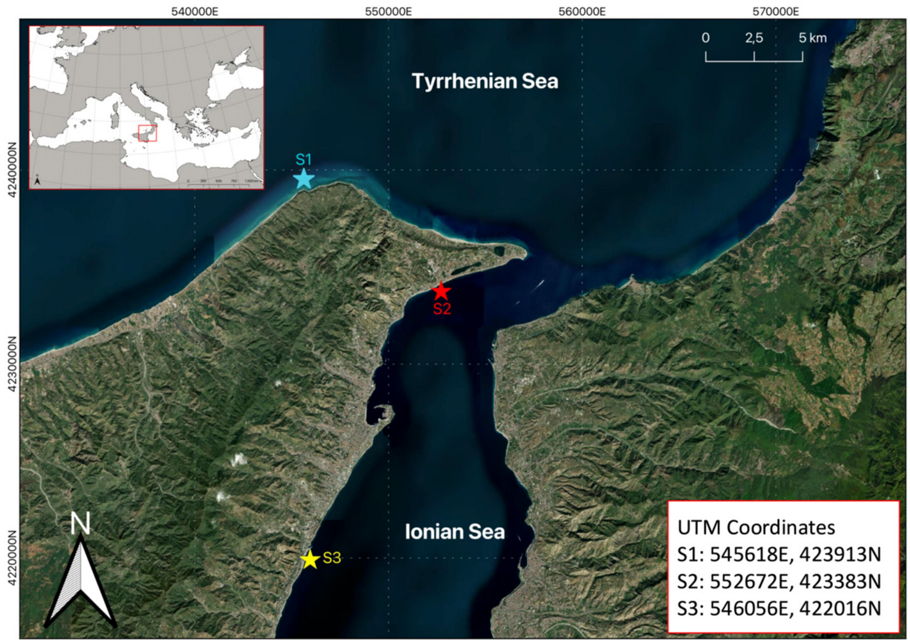

FIGURE 1
Map of the Mediterranean Sea areas investigated in this study. Report S1 related to *Octopus vulgaris* from the Tyrrhenian Sea; report S2 related to *Sepia officinalis* from the Strait of Messina; report S3 related to *Octopus vulgaris* from the Ionian Sea. Map was created with QGIS software v.3.28.1 Firenze, Geo Package ESRI satellite. The map of the Mediterranean basin in the red box was created with QGIS software v.3.28.1 Firenze, Geo Package ESRI light gray.

large population centres, coastal areas exploited by agriculture and other human activities, and the presence of an intense commercial fishing activity, characterized by the presence of a large trawling fleet and highly developed artisanal fishery (Cataudella and Spagnolo, 2011; Balassone et al., 2016; Perdichizzi et al., 2022; Foti et al., 2023). Human activities affecting the area have led to decades of uncontrolled waste dumping at sea, a massive release of contaminants and the presence of lost or abandoned fishing gear on the seabed (Ariano et al., 2019; Piazzese et al., 2019; Tamburrino et al., 2019; Rendina et al., 2020; Sciutteri et al., 2023). The Ionian Sea is a basin connecting several Mediterranean countries and is consequently characterized by heavy marine traffic that affects pollution from marine litter and pollutants released into the marine environment (Albano et al., 2021).

The fishing activity in the entire area of the Messina's Strait is historically linked to the traditional catching of swordfish and longline fishing of teleost (Sparidae, Merlucciidae, Phycidae, Trichiuridae, Scorpaenidae, etc), and other bentho-demersal resources crustaceans (mainly Palinuridae and Pandalidae), but the coastal waters are quite frequented by artisanal and recreational fishers that exert moderate anthropogenic pressure on various species of fish and Cephalopoda (Montesanto et al., 2022).

2.2 Data collection and equipment used

Data reported in the present paper have been collected by freediving and scuba underwater monitoring activities carried out monthly throughout the year 2023. The monitoring activities were aimed to assess and evaluate the presence of Recreational Fishing Marine Debris (RFMD) in coastal environments of the Strait of Messina area and surrounding zones for a different study. During the monitoring activities, occasional interaction between living organisms and marine litter, such as two reported in this study (Egis related), were documented without disturbing the animals and the surrounding environment. Video and photographic material was obtained using two GoPro Hero 11 Black and a Canon Ixus 220HS, equipped with scuba cases. One of the pieces of evidence here reported (*O. vulgaris* in the bin) derives from citizen science. In fact, a local artisanal fisherman communicated to one the authors the finding of the *O. vulgaris* specimen inside a plastic bin during the recovery of a trammel net (see results section for details).

3 Results

3.1 *Octopus vulgaris* eggs in a bin

During an artisanal fishing operation at 60 m depth on October 5th, 2023, at S1 (Figure 1), a plastic bin, 300 x 200 x 140 mm, white in colour and fractured on the top, was collected as marine litter in a net. During storage, the presence of a female specimen of *Octopus vulgaris* was noted inside the bin (Figure 2). A large quantity of eggs was subsequently observed attached to the sides and to the upper part of the bin. To avoid causing damage to the developing eggs (developmental embryos stage: XVII, according to Deryckere et al., 2020 (Deryckere et al., 2020), characterized by the presence of dark, numerous chromatophores on the posterior and anterior sides,

FIGURE 2
Octopus vulgaris specimen with eggs inside a plastic bin. Photographs obtained onboard by Marco Albano with an Apple iPhone.

absent near the eyes), only the total weight of the bin (with the specimen and eggs, and without water) and external measurements were collected. Total weight was 4500 grams. After recording the data, the bin with the *O. vulgaris* specimen protecting her eggs was released into the sea, in the same area as it was collected.

3.2 *Sepia officinalis* eggs on Egi lure

During a freediving monitoring of marine litter pollution in a coastal area (S2, Figure 1) of the Strait of Messina, Sicily, on April 4th, 2023, an Egi artificial lure commonly used in recreational fishing of cuttlefish, squid, and octopuses, was observed, and photographed. The Egi was covered by 15 *S. officinalis* eggs and entangled under a group of rocks (Figure 3) at a depth of 5.5 m. The bait was pink in colour, 90 mm long, and 14.5 g in weight (data obtained from manufacturer), with an additional weight of 5.0 g. After photographing and collecting the details, the Egi was left in the same position where it had been found. After the hatching, the Egi was again collected, to be removed from the sea.

3.3 *Octopus vulgaris* den with Egi lure

On March 10th, 2023, during some freediving monitoring activity of marine litter pollution at the site S3 (Figure 1), an orange Egi lure, 105 mm in length and 20g in weight (with an additional weight of 5g) was found at the entrance of an *O. vulgaris* den, at a depth of 8.3 m, above some stones (Figure 4). The Egi was then removed from the den opening for further analyses. The *O. vulgaris* specimen was only observed and its weight (about 1200 g) and a mantle length (about 160 mm) were estimated. No eggs were visible inside the den, even though the monitoring period coincided with the breeding season.

4 Discussion

The present study reports the evidence of previously undocumented behaviours related to the reproduction period and probably to den enhancement by two ecologically and economically important Cephalopoda species, *Octopus vulgaris* (Cuvier, 1797) and *Sepia officinalis* (Linnaeus, 1758). The use of marine litter by these two Cephalopoda species is here reported. More specifically, three types of behaviour were observed: the use of a plastic bin by *O. vulgaris* for the final act of reproduction, oviposition, and egg protection; the use of an artificial Egi bait by *S. officinalis* for egg laying; and the use of another similar artificial lure by *O. vulgaris* in apparent den enhancement. This evidence may lead to several considerations related to the Cephalopoda behaviours and interactions with marine litter, commented on below.

Octopus species use litter as shelter for several purposes, such as protection from predators, hiding, camouflage, egg laying or just because they are driven by curiosity (O'Brien et al., 2021; Freitas et al., 2022). The fact that they use litter as shelter can be considered from two diametrically opposite perspectives. The first one is related to the "widening-distribution effect" of litter presence (Mather, 1994) that acts as a pseudo-essential habitat in sandy and/or muddy substrates, improving the spatial distribution of the species. The second perspective is the potential negative effects of litter as shelter; indeed, the use of litter for sheltering and spawning by *O. vulgaris*, as here reported, can affect the health of both the specimen and the offspring (hereafter discussed).

Unlike in Freitas et al., 2022 (Freitas et al., 2022) which report that "larger species usually prefer larger shelters such as dens in rocks or reefs (Mather, 1994), and are rarely found sheltering in litter", in the present case, an adult female specimen of *O. vulgaris* selected a plastic litter bin as shelter and for eggs deposition. This highlights that *O. vulgaris* specimens, even large ones, can use any

FIGURE 3
Underwater pictures of *Sepia officinalis* eggs on a lost artificial lure (Egi). Photographs obtained underwater by Marco Albano with a Canon Ixus 220HS.

sufficiently sized object to conclude life cycle, enhancing survival and reproductive success. The choice of the bin as shelter for eggs deposition can depends on various factors such as absence of natural shelters, novelty of the object or the attractiveness of bin shape as possible site for oviposition.

Moreover, it has been reported that a recently described octopus' species, *Paroctopus ctchulu* (Leite et al., 2021), takes refuge in most cases inside marine litter (Leite et al., 2021).

Octopus vulgaris and *Sepia officinalis* reproduction is characterised by oviposition on hard structures strictly related to the benthic compartment using also artificial substrates for egg laying (Blanc and Daguzan, 1998; Bloor et al., 2013; Ganias et al., 2021; Shruti et al., 2023). This is of particular interest when considering the use of recreational artificial fishing baits, such as Egi, for egg laying. Thus, the report of these findings, both in terms of ethological and ecological aspects, is of essential importance. The use of an Egi by *S. officinalis* for egg laying is here reported for the first time, could highlight the ability of this species to recognize artificial baits as an inert object when lost in marine environments (Kelman et al., 2008). Despite this, ethological *ad-hoc* structured studies are needed to confirm this aspect. Recreational cephalopod fishing is extremely popular (Barrett et al., 2022). The most common way to catch

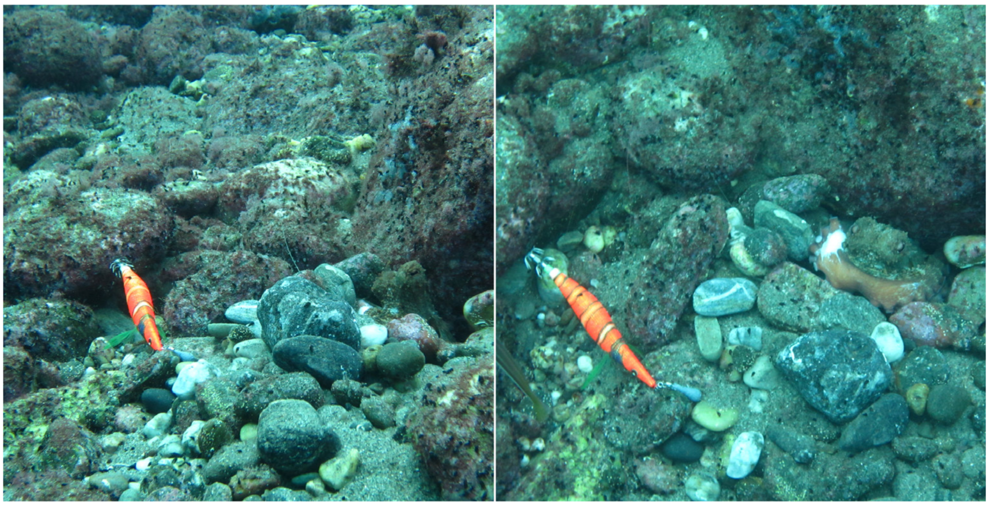

FIGURE 4
Underwater pictures of an *Octopus vulgaris* specimen inside its den with a lost artificial lure (Egi) outside the den's entrance. Photographs obtained underwater by Marco Albano with a Canon Ixus.

cephalopods from the shore is Eging fishing, a technique based on the casting and reeling of the Egi, which sinks. Once the lure has reached the bottom, it is moved by pulling with a rod, imitating a fleeing prey. If lost, the Egis can become a source of pollution, due to the potential degradation of their external coatings. The finding of an Egi with *S. officinalis* eggs attached to its external surface suggests that the female specimens recognized that the lure was not dangerous. From the position and location of the eggs that covered the Egi, it is conceivable that the *S. officinalis* manipulated the lure for egg attachment and then hid it by entangling it under a group of rocks. Similarly in this case, this highlights how reproductive of the led to the use of what is available (marine litter in this case) to complete life cycle. Although it is known that *S. officinalis*, and other cuttlefish and squid species, use artificial substrate for egg laying, what has been reported here highlights how this species adapts to the depletion of natural substrates, as well as some Gorgoniidae and Sabellidae species, algae, and others [for a more detailed list see (Guerra et al., 2016)], and to the growing presence of marine litter in our seas (Cau et al., 2019, 2024).

Based on the above considerations, it can be assumed that the reproductive stimulus acts as a driving force for adaption of marine animal species to continuously changing environments (Eleutério, 2012), and specifically for the cases reported in this study, how wild Cephalopoda have leveraged non-natural objects as a resource for the oviposition.

The recognition of the Egis by Cephalopoda species is also highlighted by the last piece of evidence reported in this study, specifically where a specimen of *O. vulgaris* probably used one of these lures to enhance a natural rocky den. *Octopus vulgaris*, like other Octopoda, has a peculiar behaviour in enhancing the den in which it spends most of its life, especially during daytime. The dens of *O. vulgaris* can vary (Katsanevakis and Verriopoulos, 2004b; Guerra et al., 2014), but almost all share an enrichment at the entrance, characterized by the presence of hard objects of various shapes, origins, and composition. It would appear that *O. vulgaris* does not show a specific preference for one kind of object, demonstrating a behavioural plasticity that springs from innate responses, individual experiences, and the ability to recognize innocuous objects (Mery and Burns, 2010; Florida et al., 2018). These hard objects are also used to close and camouflage the den entrance when the octopus retreats inside. In the case of Egi, the recognition of the lure as harmless offers an assumption of high importance. The lost Egi used by a specimen of *O. vulgaris* for den enhancing may suggest that the animal manipulated and exploited it for its behavioural habits, highlighting the ability to recognise objects as innocuous. The use of artificial substrates for shelter, and marine litter in general for den construction, is reported for several octopus' species (Freitas et al., 2023), and may result from the depletion of hard objects of natural origin, empty shells *in-primis*, due to their removal by tourists for souvenirs (Kowalewski et al., 2014). This behaviour may also depend on the presence and increasing abundance of marine litter, to which wild species are increasingly adapting (Briffa et al., 2024).

Finally, the potential effects of plastic substrates on both specimens and their offspring should be considered. The objects used by the species mentioned in this paper can be included in the macro-category of marine litter and as is well known, plastics is composed of and/or adsorbs/releases chemicals (Avio et al., 2017) that are potentially toxic to the specimens it encounters. This is especially true in the cases of the present paper in which *O. vulgaris* and *S. officinalis* used the litter objects for oviposition and apparent den enhancement (only *O. vulgaris*). The manipulation and use of the bin and Egis may result in the ingestion of small fragments by the specimens and/or have toxic effects on the offspring (Ganias et al., 2021; Pedà et al., 2022).

In this context, there is evidence in literature regarding the effects of micro-litter (e.g. microplastics) on the ecological performance of marine organisms through the analysis of functional traits. These studies have shown that exposure to microplastics in different taxa can moderately alter some functional traits and have repercussions on the demographic traits of a population, as well as at higher hierarchical levels, compromising ecosystem functionality (Berlino et al., 2021). To date, however, the effect of macro-litter on the performance traits and possibly on the fitness of marine organisms has been neglected. Some authors have shown how macro-litter can influence the abundance of some benthic organisms, which show variable responses including "positive", negative, or neutral effects (Clemente et al., 2018; Battaglia et al., 2019; Carugati et al., 2021), others describe the potential role of macro-litter on the dispersal of organisms, including rafting transport of alien and/or invasive species (Rech et al., 2018; Mghili et al., 2023). Regarding oviposition events, there is scanty information on the potential effects on offspring. Regardless of the potential negative effects on the species and offspring, what is here reported may lead to the "paradoxical benefit" of litter in cases where artificial objects represent pseudo-essential habitats for various phases of marine fauna life cycles (Spinelli et al., 2020; Carugati et al., 2021).

In conclusion, the present work provides new data that broadens the spectrum of knowledge regarding interactions between cephalopods, specifically octopus and cuttlefish, and litter present in marine environments. The three reported episodes are the result of citizen science (from an artisanal fishing operation) and freediving monitoring of marine litter pollution (for *ad-hoc* structured study) in some areas along the coastline of Messina. It is therefore clear that direct marine *in-situ* observation can be useful to understand how a wide range of organisms may interact with marine litter in the natural environment, adapting to its presence, and, in some cases, modifying their habitual behaviours.

Based on observations, and as seen in previous works (Ganias et al., 2021; Freitas et al., 2022, 2023; Pedà et al., 2022), marine litter of different shape, colour and size is used by cephalopods as substrates for oviposition and to increase the degree of protection of their dens. The remarkable intelligence of these creatures (Mather, 1994; Amodio et al., 2019; Chung et al., 2022; Hoffmann, 2022) enables them to recognize and manipulate marine litter according to their needs, as if they were natural objects. Such adaptations involve not only cephalopods but also many other benthic organisms, which over time have managed to adapt and turn the issue of marine litter contamination of marine environment into objects/structures to be exploited (Briffa et al., 2024; Jagiello et al., 2024).

This represents, paradoxically, a resource for these organisms, as marine litter can increase the structural complexity of the seafloor, providing some faunal species with additional supports to complete their life cycle. Increasing the structural complexity of the seafloor is at the base of the use of artificial reefs for the restoration of degraded marine ecosystem (Bracho-Villavicencio et al., 2023). Artificial reefs (ARs) are benthic structures used for protection and/or enhancement of local species diversity and abundances [Seaman and Lindberg, 2009; United Nations Environment Programme (UNEP), 2009]. In contrast to ARs, which should be made by inert materials, marine litter objects are variously composed, and it is necessary, through new *ad-hoc* structured studies, to further investigate and evaluate the possible effects, still largely unknow, caused by marine litter (plastic composed one *in-primis*). Coming into direct contact with organisms and eggs, marine litter may interfere with their health, with probable medium- to long-term effects impacting the entire trophic web. This becomes even more relevant regarding semelparous species, as the eggs deposition substrate is of crucial importance for both the success of the reproductive event and the offspring.

The authors recommend the reader to consider that this study is based on individual and occasional behavioural observations. Therefore, it should be considered a first step in monitoring such findings with a further monitoring effort by the scientific community.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because the study was based only on diving observation, without any sampling or disturbance for the living animals reported.

Author contributions

GC: Conceptualization, Investigation, Writing – original draft. MA: Conceptualization, Data curation, Methodology, Validation, Writing – review & editing. CD'I: Data curation, Formal Analysis, Visualization, Writing – review & editing. SF: Writing – review & editing. DF: Data curation, Methodology, Writing – review & editing. NS: Project administration, Resources, Writing – review & editing. SS: Conceptualization, Validation, Writing – review & editing.

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Conflict of interest

GC, SS, and NS are the co-founders of Sea in Health and Life srl.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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