



In an octopus's garden in the shade: Underwater image analysis of litter use by benthic octopuses

Tainah B.N. Freitas^a, Tatiana S. Leite^b, Bruna de Ramos^c, Anna di Cosmo^d, Maíra C. Proietti^{a,*}

^a Programa de Pós-graduação em Oceanografia Biológica and Projeto Lixo Marinho, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, Avenida Itália Km 08, Rio Grande, RS, Brazil

^b Laboratório de Métodos de Estudos Subaquáticos e Cefalópodes, Departamento de Ecologia e Zoologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

^c Departamento de Oceanografia, Universidade Federal de Pernambuco - UFPE, Recife, PE, Brazil

^d Department of Biology, University of Napoli Federico II, Complesso Universitario Monte Sant' Angelo, Via Cinthia, 80126 Napoli, Italy

ARTICLE INFO

Keywords:

Marine debris
Octopuses
Shelter selection
Impacts
Citizen science
Cephalopoda

ABSTRACT

Benthic octopuses have been widely documented in artificial shelters for decades, and this use is apparently increasing. Despite any possible positive effects, the use of litter as shelter could have negative implications. In this work, we aimed to elucidate the interactions of octopuses with marine litter, identifying types of interactions and affected species and regions. To achieve this, we obtained 261 underwater images from 'citizen science' records, and identified 8 genera and 24 species of benthic octopuses interacting with litter. Glass objects were present in 41.6% of interactions, and plastic in 24.7%. Asia presented the highest number of images, and most records were from 2018 to 2021. Citizen science provided important evidence on octopus/marine litter interactions, highlighting its value and the need for more investigations on the subject. This information is fundamental to help prevent and mitigate the impacts of litter on octopuses, and identify knowledge gaps that require attention.

1. Introduction

Interactions of marine fauna with litter are widely documented and include ingestion (Brandão et al., 2011; De Stephanis et al., 2013; Rizzi et al., 2019), entanglement (Barreiros and Raykov, 2014; Moore et al., 2013; Wegner and Cartamil, 2012) and habitat change (O'Hanlon et al., 2019; Smith, 2012; Uhrin and Schellinger, 2011). Additionally, litter can be used as substrate for colonization by sessile organisms (Lacerda et al., 2020; Santín et al., 2020), and as shelter for a variety of mobile organisms such as hermit crabs, sea urchins and cephalopods (Barreiros and Luiz, 2009; Barros et al., 2020; Heery et al., 2018). Some animals have also been recorded actively covering their bodies with materials – including litter – present in the environment for protection. For instance, Barros et al. (2020) describe that the sea urchin *Lytechinus variegatus* covers itself with plastic and metal debris as well as organic materials; the authors highlight that this camouflage can be effective if litter is prevalent in benthic environments.

Octopuses are considered intelligent and charismatic animals (Iglesias et al., 2014), and are therefore common subjects in neuroscience

and behavior studies (Di Cosmo et al., 2018, 2021; Medeiros et al., 2021), as well as in underwater photography and videos around the world. They are also used as ornamental species in aquariums (Vidal et al., 2014) and have appeared in movies (e.g. Academy Award-winning "My Octopus Teacher") and on-line plays (e.g. "Temple du Present – Solo pour Octopus"). Octopuses can also be portrayed in mythological and monstrous forms such as the Kraken, which also demonstrates how humans are fascinated with these organisms. A characteristic behavior of benthic octopuses is to avoid predation by actively selecting and modifying shelters in the substrate, where they remain most of the time (Katsanevakis and Verriopoulos, 2004; Maselli et al., 2020). Females also deposit their eggs in the chosen shelters, and a successful choice can affect their fertility (Iribarne, 1990), being therefore crucial to the survival and reproductive success of these organisms.

Bivalve and gastropod shells are commonly used as refuge and egg-laying sites by benthic octopuses (Narvarte et al., 2013), especially by small animals that live in sandy and muddy habitats where shelters in rocks and reefs are scarce (Iribarne, 1990; Katsanevakis and Verriopoulos, 2004; Mather, 1982a). On the other hand, shell removal due to

* Corresponding author.

E-mail addresses: tati.polvo@gmail.com (T.S. Leite), icosmo@unina.it (A. di Cosmo), mairaproietti@gmail.com (M.C. Proietti).

tourism and ornamental purposes has increased in the past decades, reducing its availability in marine ecosystems (Kowalewski et al., 2014; Souto Alves et al., 2006). Shelter availability affects the distribution pattern of several octopus species: for instance, the pygmy octopus *Octopus joubini* only occurs in areas where shells are available to hide in, and patterns of individual distribution seem to depend on access to shelter. Empty shells also determine the spatial distribution of the two-spot octopus *Octopus bimaculoides* (Ambrose, 1982), and individuals compete for the highest quality shelters, preferring those with small entry size (Cigliano, 1993). Shelter is a key factor in the life cycle of the ruby octopus *Octopus rubescens*, (Iribarne, 1990), and glass bottles allow them to occupy soft sediment areas where natural dens are scarce (Anderson et al., 1999). In addition, *Paroctopus cthulu*, a newly described species of pygmy octopus from Brazil, has until the present only been observed sheltering in litter, especially metal beverage cans (Leite et al., 2021).

As natural shelters become scarcer in the marine environment, finding high quality shelter could represent a possible advantage for octopuses. For instance, the coconut octopus *Amphioctopus marginatus* has figured out a way to make their shelter portable by developing a type of locomotion called “stilt-walking”, in which the organism carries its shelter while moving. This behavior likely evolved using large empty bivalve shells (Finn et al., 2009), but is currently more commonly observed with coconut shell halves or other human litter, and allows them to forage more safely when no other shelters are available. Additionally, benthic octopuses use litter as a tool to modify shelters and increase their protection, or to mark their territory. For example, *O. vulgaris* can remove stones and sand from their dens, adding other items such as empty shells and litter to the opening of the shelter (Mather, 1994). The shelter opening is the part most vulnerable to predator attacks, and the behavior of hiding in narrow refuges provides an adaptive advantage to octopuses, since they can pass through tiny spaces unlike their predators and competitors of similar sizes.

Despite these descriptions, few studies have focused on the interactions between cephalopods and marine litter, and scientific information on this subject has scarcely been updated over the last decades (Aronson, 1986; Heery et al., 2018; Iribarne, 1990; Katsanevakis and Verriopoulos, 2004; Mather, 1994; Voight, 1992). In this scenario, citizen science emerges as a valuable tool to better understand these interactions. Defined as the “work undertaken by civic educators together with civilian communities to advance science” (Ceccaroni et al., 2017), citizen science stimulates a scientific mentality and encourages community engagement, helping society to work on complex issues, aggregating global information, and filling knowledge gaps (Dickinson et al., 2012). Since divers from all over the world are increasingly interested in cephalopods, there are numerous photographic and audiovisual records of these animals online; among these records, several involve benthic octopuses interacting with litter. Thus, the involvement of divers and evaluation of their images can provide important information about the interactions between cephalopods and marine litter.

In this study, we used underwater images obtained online to answer the following questions concerning the interaction between octopuses and marine litter: (1) What are the types of interactions between octopuses and litter? (2) Do octopuses interact more frequently with a certain type of material? (3) Which species present the greatest number of records? (4) Where are most interactions recorded? (5) Are records of such interactions increasing over time? We hypothesized that benthic octopuses would interact mainly with plastic items since this material is predominant in marine environments, and that small species that commonly shelter in mollusks shells would have more interaction records than those that use holes in rocks and reefs. By answering the above-cited questions, we obtained important information to help prevent and mitigate the impacts of litter on octopuses, and identify knowledge gaps that require attention in the future.

2. Methodology

To evaluate the interactions of benthic octopuses with marine litter, we compiled photos and videos through underwater image databases (Shutter Stock, Ocean Wide Images, Alamy, Science Photo Library, Nature Picture Library, and Global Biodiversity Information Facility), and images available on Facebook and Instagram social media platforms. The authors of records that presented octopuses interacting with litter were contacted to ensure free access and use of images. We also used the first level of a citizen science approach (Haklay, 2012) and requested that the participants of the following groups send us their records: Cephalopod Appreciation Society, Octonation, Marine Life Behavior, “Octopi...This group”, Le Jardin de Poulpito, Voice of the Ocean, OceanShutter, Caters News, MarineBio.org, Observations de Céphalopodes en France, UK Cephalopod Reports, Avistamiento de Cefalopodos Espanha, Blue Ocean Divers, Cyprus Marine Sea Life, Underwater Photography in Greece, Underwater Photographers, and Scuba Divers Life. In addition, we carried out a national and international campaign through the Projeto Cephalopoda social media (Instagram and Facebook) and the Cephalopod International Advisory Council (CIAC) email group requesting collaboration via records of interactions. Image databases were searched until December 2020, but one additional photo of an octopus found sheltering in a battery (Fig. 1e) was received in April 2021 and included in the data due to its novelty. However, this record was not included in the temporal analysis since we did not systematically search for images in 2021.

All images included in our evaluation allowed the identification of the organisms and litter involved, and focused on interactions between benthic octopuses and litter. For each selected image, we collected information regarding the octopus's taxa, type of interaction, litter characteristics, depth, location of the record, and date. We also considered additional information on the website, and any comments provided by the photographers. For situations in which there was no information about the registered species, we used an identification key (Jereb et al., 2013) and an expert researcher to reach the lowest possible level of identification and to evaluate how species naturally use shelters. The interactions observed in the selected underwater images were classified into five categories: i) sheltering (Mather, 1982a) – when octopuses were inside the litter, either completely inside or with their eyes/head out the opening; ii) out of the litter – when octopuses were next to the litter opening, but with their whole body outside it; iii) on top of the litter – when octopuses were on top of litter; iv) burrowing (Hanlon and Messenger, 1996) – when octopuses were among or under litter in order to hide; and v) stilt-walking (Finn et al., 2009) – when octopuses were moving and carrying their shelter. Sheltering, burrowing, and stilt-walking categories have already been described in scientific literature, while out of the litter and on top of the litter categories were created based on the observations of interactions with litter. When present, egg deposition was also recorded.

We classified the litter used by octopuses in terms of material (cloth, glass, metal, miscellaneous, plastic, rubber, organic, undetermined) and type (see Table 2 in the results section for a detailed description of type categories) according to GESAMP (2019), using the classification suggested by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). We highlight that despite being organic materials, coconut shells, timber, and food were considered human-originated litter, as they are modified by a series of mechanical/chemical processes and commonly enter the ocean via human activities. Item color (according to the 12 basic color terms of the Inter-Society Color Council National Bureau of Standards/ISCC-NBS) and transparency, as well as incrustations, were also recorded. Litter was considered transparent when it was possible to see the animal through it. Although few records presented depth information, we had access to photos taken in deep regions (>100 m) of the Mediterranean with a ROV. Considering the depths at which scuba dives occur (generally up to a maximum of 30-40 m), we assumed these records as “shallow”, and classified those taken

with the ROV as “deep”.

To test for significant differences in the materials used by benthic octopuses, an analysis of proportions using a chi-squared goodness of fit test was done in the statistical environment R 3.2.0. The same test was also used to assess if the number of records was different between species that naturally use mollusks shells and those that shelter in holes in rocks and reefs, in which we determine whether a variable is likely to come from a specified distribution or not. Therefore, we tested if the proportions of $x_1, x_2, x_3, \dots, x_n$ occurrences were equal; differences between groups were considered significant if $p < 0.05$. The absolute and relative frequencies of litter material, type, color, and type of interaction were estimated. In terms of record dates, records were represented yearly, and image locations were grouped into continents and represented as absolute frequencies. To summarize data on the litter materials that octopuses interact with worldwide, we generated a map

using QGIS software version 3.16 (QGIS Development Team, 2020).

3. Results

We compiled 261 underwater images of octopus species interacting with marine litter, including videos ($n = 15$; 5.8%) and photographs ($n = 246$; 94.2%). It was possible to identify 75.0% of the authors of the records, and the images were taken by 113 photographers. Most photographers contributed with one record ($n = 82$; 72.6%), while 31 (27.4%) sent two or more images; the photographer with most images took 10 of the records. Regarding the origin of the records, the main sources were social media such as Facebook and Instagram ($n = 143$; 54.8%), followed by underwater image databases ($n = 76$; 29.1%) and websites ($n = 12$; 4.6%). We also received 28 images (10.7%) from researchers via e-mail and collected two records from a scientific paper



Fig. 1. Examples of interactions of benthic octopus species with marine litter, observed in underwater images from around the world. a-b) *Octopus vulgaris* sheltering in a broken glass bottle and interacting with a whole one (photos: John Paul Meillon); c) *Amphioctopus marginatus* carrying two plastic items while ‘stilt-walking’ (photo: Serge Abourjeily); d) *Macrotritopus cf defilippi* inside two plastics cups (photo: Claudio Sampaio); e) *O. americanus* found sheltering in a battery (photo: Caio Salles); f-g) *Paroctopus cthulu* found inside beverage cans, including a female with eggs (photos: Edmar Bastos); h) *O. vulgaris* in a rusted metal pipe (photo: Marco Panico); i) *A. marginatus* sheltering in a metal pot and using litter and mollusk shells to increase protection (photo: Federico Betti).

that investigated disruptions in ecological processes of reef systems caused by marine litter (de Carvalho-Souza et al., 2018). In addition, we received or found 19 images that were not included as data in this research, since they documented a different cephalopod group than the target one, or depicted an interaction in an artificial environment. These images were of: cuttlefish individuals swimming/resting on debris at the seafloor; squid and cuttlefish eggs deposited in abandoned fishing gear; and octopuses sheltering in aquaculture systems/aquariums or interacting with divers' cameras.

The main interaction recorded was sheltering ($n = 178$; 68.2%), followed by on top of the litter ($n = 30$; 11.5%), burrowing ($n = 26$; 10.0%), out of the litter ($n = 18$; 6.9%) and stilt-walking ($n = 9$; 3.5%) (see examples in Fig. 1). Each image presented only one type of interaction; however, octopuses were documented interacting with more than one type of litter in some records, totaling 279 marine items (Fig. 2). It was possible to identify 83.1% of animal taxa in the records, with a total of 8 genera and 24 species (Table 1). The coconut octopus *Amphioctopus marginatus* was the most common species observed interacting with litter (42.5%). Seven of the sheltering records were of female octopuses with eggs (Fig. 1g); the species registered with eggs were *A. marginatus* ($n = 2$), *Paroctopus cthulu* ($n = 2$), *Amphioctopus siamensis* ($n = 1$), *Octopus maya* ($n = 1$) and *Octopus insularis* ($n = 1$). Benthic octopuses that naturally use mollusk shells as shelter ($n = 128$; 49.0%; $p < 0.01$) showed significantly higher number of interactions with marine litter than octopuses that do not use mollusk shells as a natural habitat ($n = 89$; 34.1%).

Regarding the materials that composed the litter observed in all underwater images, glass was the most frequent (41.6%), followed by plastic (24.7%), metal (17.6%), organic (8.6%), miscellaneous (2.9%), undetermined (2.2%), rubber (1.4%) and cloth (1.1%). All the identified materials were used as shelter by benthic octopuses, but octopuses interacted more frequently with glass items, especially for sheltering and out of the litter ($p < 0.05$) (Fig. 2). Meanwhile, plastic items were more common in on top of the litter interactions (43.3%) and burrowing (34.6%) ($p < 0.05$). Only organic (45.5%), plastic (45.5%) and glass (9.0%) items were recorded in the stilt-walking interaction. Several types of human-made objects were used as shelters, but the most frequent were glass beverage bottles (32.0% of sheltering records), glass jars (9.0%), metal beverage cans (8.3%), and coconut shells (7.9%)

(Table 2). When sheltering in marine litter, we observed octopuses using other litter items such as metal and plastic caps/lids, glass and plastic fragments, a metal spoon, and coconut shells as camouflage; when sheltering between rocks or in sand burrows, they were recorded using larger debris such as glass bottles, metal cans, plastic bags, and part of a surfboard to camouflage their shelters. Other identified litter types are listed in Table 2.

Species that use mollusk shells interacted more with glass ($n = 56$; 43.7%), plastic ($n = 34$; 26.5%), metal ($n = 27$; 21.1%), organic ($n = 21$; 16.4%), cloth ($n = 2$; 1.6%), and undetermined ($n = 2$; 1.6%). Large species that commonly use larger shelters such as rocks and reef dens interacted more with glass ($n = 38$; 42.7%), plastic ($n = 27$; 30.3%), metal ($n = 17$; 19.1%), miscellaneous ($n = 5$; 5.6%), rubber ($n = 3$; 3.3%), undetermined ($n = 3$; 3.3%), organic ($n = 1$; 1.1%), and cloth ($n = 1$; 1.1%). Although both groups interacted more frequently with glass and plastic objects, it was not possible to establish any preference pattern.

When assessing the transparency of the items used as shelter by octopuses, 37.4% were translucent, while 62.6% were opaque, and 51.9% showed incrustations, indicating long permanence in the marine environment. In terms of color, brown (23.4%) and transparent (22.6%) litter were the most recorded. Among the observed bottles and cans, 9.6% were damaged, broken or rusted. We identified two product names within the 279 items: Coca-Cola ($n = 7$) and Sprite ($n = 2$).

In relation to the time period of interactions, it was not possible to identify the date of 49 underwater images. Of those identified, the first was taken in 2003, and the number of images increased progressively over the years. The temporal tendency line of citizen records increased in a logarithmic pattern, and the period from 2018 to 2021 included 49.5% of the images (Fig. 3). In terms of locations, images were from Indonesia, Philippines, Malaysia, Thailand, Australia, New Zealand, England, France, Cyprus, China, Turks and Caicos Islands, Brazil, United States, Spain, Egypt, Greece, Mexico, Italy, and Malta. Asia was the continent with the most underwater images of octopus/litter interactions ($n = 114$) – with 94 of the 111 records of *Amphioctopus marginatus* occurring there – followed by Europe ($n = 36$) and Oceania ($n = 24$); records for Central ($n = 1$), North ($n = 16$) and South America ($n = 15$) were low (Fig. 4). It was not possible to identify the location of 55 images.

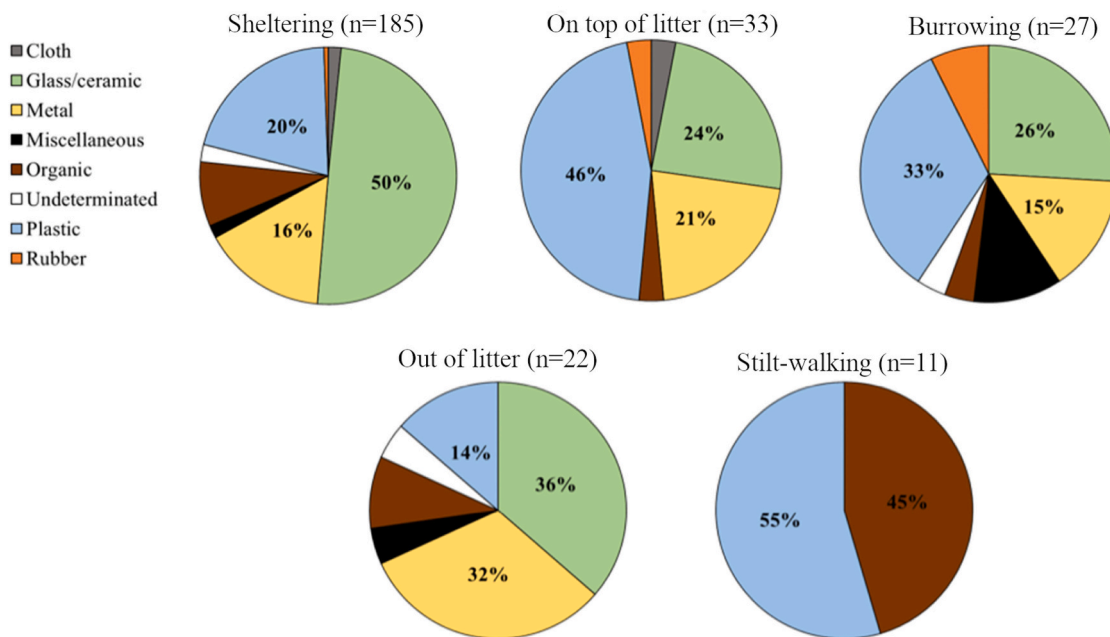


Fig. 2. Absolute frequency of occurrence of materials that composed litter items found in association with benthic octopuses for each type of interaction recorded in underwater images.

Table 1

Octopus species and genera identified in online underwater images interacting with marine litter, with indication of taxa, number of records (N) for each species, if the species commonly uses mollusk shells as shelter, and known regions/countries of occurrence (based on Jereb et al., 2013; Leite et al., 2021).

Taxa	N. records	Mollusk shells	Regions/countries of known occurrence
<i>Amphioctopus aegina</i>	1	Yes	Indian Ocean (China, Taiwan, Malaysia, Indonesia, India, Philippines)
<i>Amphioctopus arenicola</i>	1	Yes	Northeastern Pacific, Hawaiian Islands Archipelago
<i>Amphioctopus burryi</i>	3	Yes	Gulf of Mexico, tropical Western Atlantic (North Carolina to northern Brazil), tropical Eastern Atlantic (west Africa)
<i>Amphioctopus marginatus</i>	111	Yes	Tropical Indian Ocean (South Africa to Red Sea, India, Taiwan, Philippines, Japan, northeastern Australia)
<i>Amphioctopus siamensis</i>	3	Yes	Andaman Sea, Gulf of Thailand
<i>Amphioctopus sp</i>	2	Yes	Tropical and subtropical waters of all oceans
<i>Callistoctopus macropus</i>	1	No	Mediterranean Sea, eastern Atlantic Ocean to Dakar, Senegal
<i>Callistoctopus ornatus</i>	1	No	Tropical Indian and western and central Pacific Oceans
<i>Hapalochlaena lunulata</i>	2	No	Indonesia, Philippines, Papua New Guinea, Vanuatu, Solomon Islands
<i>Hapalochlaena sp</i>	2	No	Central Indo-West Pacific Ocean, north to Japan, south to southern Australia
<i>Macroctopus maorum</i>	1	No	New Zealand, southern and western Australia
<i>Macrotritopus defilippi</i>	3	No	Mediterranean Sea, northeastern Atlantic Ocean
<i>Octopus americanus</i>	1	No	Western central Atlantic Ocean (South Carolina to Venezuela)
<i>Octopus berrima</i>	2	No	Australia
<i>Octopus bimaculoides</i>	1	No	Northeast Pacific Ocean (Southern California to Mexico)
<i>Octopus cyanea</i>	2	No	Tropical Indo-West Pacific Ocean (eastern Africa to Hawaii, southern Japan to northern Australia)
<i>Octopus insularis</i>	3	No	Brazil (northeastern mainland and oceanic islands)
<i>Octopus maya</i>	1	No	Gulf of Mexico
<i>Octopus pallidus</i>	1	No	Southeast Australia
<i>Octopus rubescens</i>	3	Yes	Gulf of California, Pacific Mexico to Gulf of Alaska
<i>Octopus salutii</i>	4	No	Mediterranean Sea, northeastern Atlantic Ocean
<i>Octopus sinensis</i>	1	No	Japan
<i>Octopus tetricus</i>	20	No	Temperate eastern Australia, northern New Zealand
<i>Octopus vulgaris</i>	40	No	Mediterranean Sea, North Atlantic Ocean
<i>Octopus sp</i>	2	No	Temperate waters of all oceans
<i>Paroctopus cthulu</i>	4	Yes	South and southeastern Brazil
<i>Thaumoctopus mimicus</i>	1	No	Tropical Indo-West Pacific, from north-east Australia and New Caledonia to the Philippines, west to the Red Sea
Unidentified	44	-	-

Interactions such as competition and predation of octopuses on other organisms related with litter were also recorded. Two images showed an octopus inside its artificial shelter with captured prey (a hermit crab and a fish); five images showed benthic octopuses competing with other octopuses ($n = 4$) and a hermit crab ($n = 1$) for artificial shelters. Underwater images also provided records of a range of bivalve shells, stones and marine litter being used to camouflage the opening of the shelters chosen by animals (see Fig. 1 – i). Regarding depth, most of the records were in shallow depths (i.e. scuba diving depth; $n = 271$;

Table 2

Marine litter classification used in the evaluation of cephalopod/litter interactions, with number of observations of each material and type in underwater images. Classification followed the CSIRO category list in GESAMP (2019).

Material	Type	Num. Observations
Cloth	Clothing/shoe	2
	Teddy bear	1
Total (%)		3 (1.1%)
Glass	Beverage bottle	80
	Dish	2
	Fragment	8
	Jar/pots	25
	Souvenir	1
Total (%)		116 (41.6%)
Metal	Battery	1
	Beverage can	23
	Bucket/crate	2
	Cutlery (spoons, forks, knives)	1
	Fish hook	2
	Food can/tin	8
	Lid/cap	4
	Pipe	5
	Other metal items	3
Total (%)		49 (17.6%)
Miscellaneous	Brick/cement	6
	Car/bicycle/boat parts	1
	Leather	1
Total (%)		8 (2.9%)
Organic	Coconut shell	22
	Food	1
	Wood/timber	1
Total (%)		24 (8.6%)
Plastic	Beverage bottle	9
	Bottle cap/lid	6
	Bucket/crate	3
	Fishing line	3
	Food container	15
	Fragment	7
	Gloves	1
	Glow stick	2
	Golf ball	1
	Masks	2
	Net	3
	Other type of bottle	8
	Pipe	6
	Plastic bag	2
	Surfboard	1
Total (%)		69 (24.7%)
Rubber	Thong/shoe	1
	Tire	3
Total (%)		4 (1.4%)
Undetermined	Undetermined	6
Total (%)		6 (2.2%)
TOTAL		279

98.1%). For images taken with a ROV in deep regions of the Mediterranean Sea, five records (1.9%) were available for octopuses between 100 and 400 m. It was possible to observe *Octopus vulgaris* ($n = 1$) among fishing lines off the coast of Italy, and *Octopus salutii* ($n = 4$) within glass/ceramic fragments, and close to a fishing hook, ropes, and other unidentified litter off the coast of Italy and France (see examples in Fig. 5).

4. Discussion

Benthic octopuses have been documented for decades using litter for sheltering and laying their eggs; however, this is the first study to systematically evaluate and characterize the different uses of litter by these animals using underwater images. Our results showed an increase in octopus/litter interaction records, possibly due to the higher abundance and attention of this type of pollution in marine ecosystems. However, this increase could also be due to the lower costs of digital photos and higher availability of underwater imaging equipment nowadays. In any case, considering that adequate shelter choice is vital for the survival

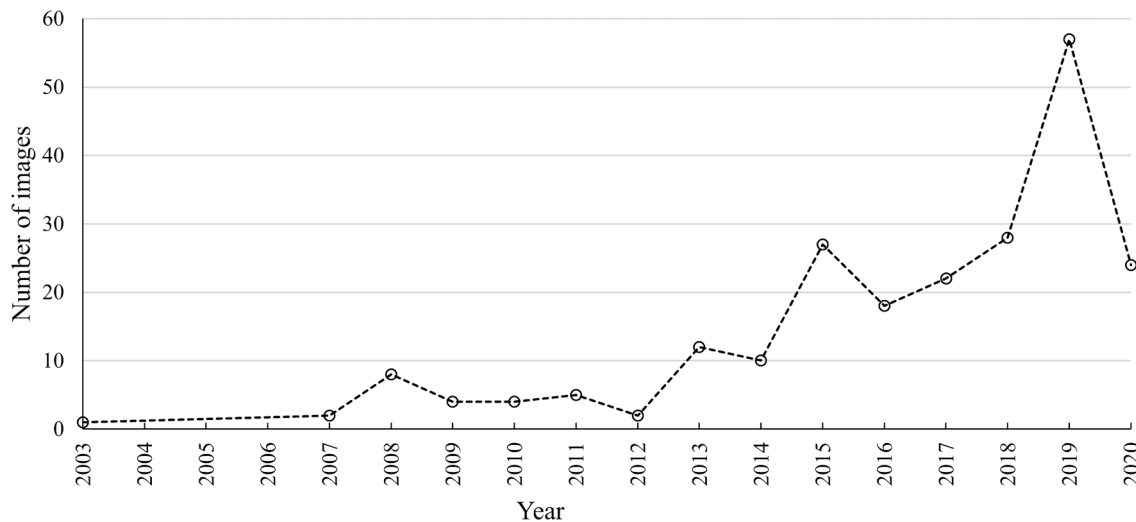


Fig. 3. Number of underwater image records of interactions between octopuses and marine litter recovered from worldwide registers over the years (2003–2020).

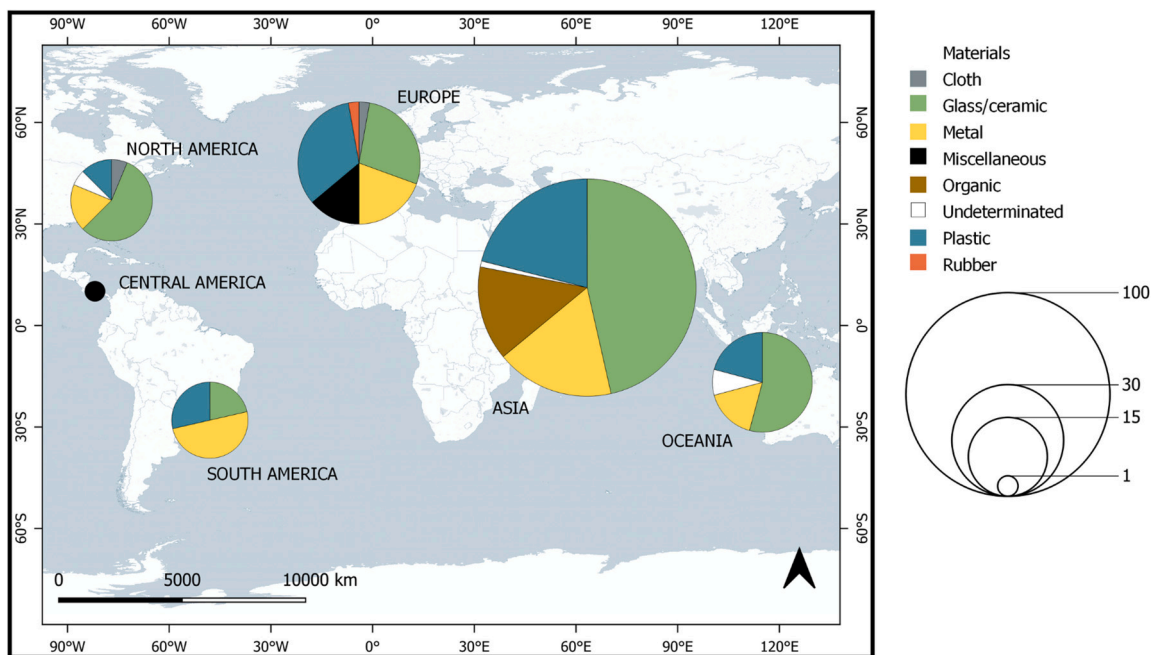


Fig. 4. Relative frequency of underwater image records of interactions between octopuses and marine litter, according to continents and with indication of litter material.

and reproductive success of benthic octopuses, litter can provide potential advantages, such as allowing them to occupy areas with limited natural shelter. For example, the availability of marine litter affects the distribution patterns of the common octopus *O. vulgaris* on soft sediments (Katsanevakis and Verriopoulos, 2004), and the giant Pacific octopus *Ectopoctopus dofleini* occurs more commonly in areas with higher amounts of marine litter available to use as shelter (Heery et al., 2018).

As mentioned above, at first sight the use of litter as shelter in environments with low availability of natural seashells may offer an advantage/protection for these organisms. However, associations with marine litter can have multiple implications, and any apparent positive effect could also have several detrimental and indirect consequences. Our evaluation showed that benthic octopuses use litter of different materials, including glass, metal, ceramic and plastic; sheltering and laying eggs in plastic litter, for instance, may result in the exposure of the animal to toxic components, since plastics contain toxic chemical

additives in its composition and can also adsorb contaminants present in the environment (Avio et al., 2017). Some types of litter such as tires can accumulate contaminants such as heavy metals, which can be even more detrimental for these animals (Collins et al., 2002; Kwon and Kim, 2015). For instance, we posteriorly observed a record of an octopus sheltering in a battery (see Fig. 1 – e), a very polluting type of debris. Additionally, the constant removal of litter from the environment through cleanups could represent a threat to organisms or populations that frequently use litter as shelter, and broken or damaged glass and metal items could physically injure octopuses (Leite et al., 2021). However, the small percentage of damaged objects chosen by octopuses as shelter in the evaluated images may indicate a preference for high-quality materials (i.e. objects that are in their original state of manufacture, which means not broken or rusty).

We hypothesized that octopuses would interact mainly with plastic materials since this type of litter is highly abundant in marine

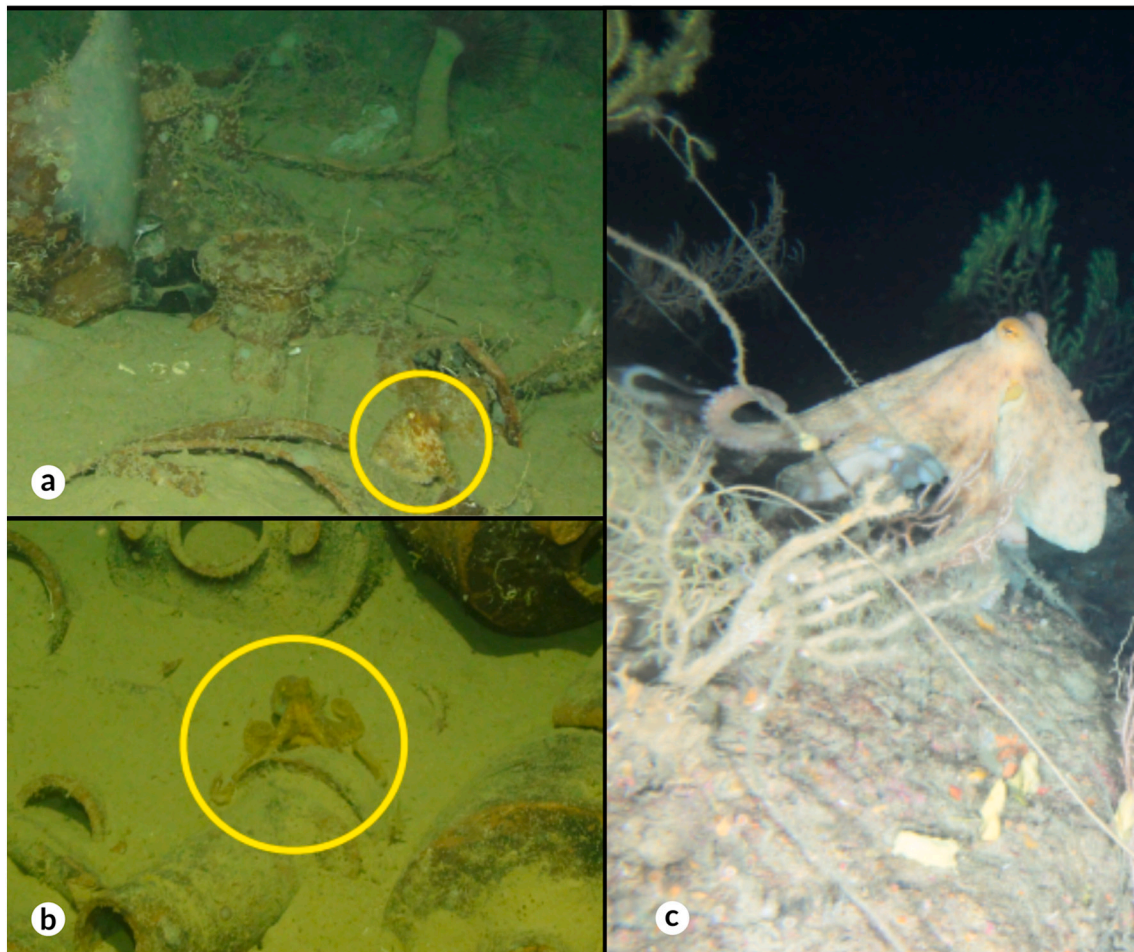


Fig. 5. Deep-sea octopuses interacting with marine litter, registered through underwater ROV surveys. a) *Octopus salutii* within glass/ceramic fragments at 400 m depth, west of Gorgona Island, Tuscany, Italy; b) *O. salutii* within glass/ceramic fragments at 325 m, east of Macinaggio, Corse, France; c) *O. vulgaris* among stuck fishing lines at 100-180 m depth in Banco di Graham, Sicily, Italy. Yellow circles indicate animals. Photos: Marzia Bò. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ecosystems (Galgani et al., 2021). However, underwater images revealed that octopuses more frequently used glass items than plastic ones, mainly for sheltering. This could be due to the fact that glass presents higher density ($\sim 2500 \text{ kg/m}^3$) than seawater ($\sim 1020 \text{ kg/m}^3$) and usually sinks to the seafloor, being available in higher numbers for use by benthic species. Meanwhile, the most common plastic polymers found in the ocean (polyethylene, polypropylene and polystyrene; Erni-Cassola et al., 2019) have lower density (in general $< 1000 \text{ kg/m}^3$) than seawater and can remain at the surface and water column for a long time. Furthermore, plastic objects tend to fragment in marine ecosystems due to prolonged exposure to UV light and physical abrasion through wave action (Hollman et al., 2013), and may therefore not be adequate for use as a shelter. It can also be theorized that glass objects have more suitable shapes to be used as shelters, since bottles and jars are common glass items found at the seafloor (Canals et al., 2021; Galgani et al., 2000); also, the texture of glass may be more similar than plastic to the internal texture of seashells, contributing to its use as shelter. However, plastic was more common than glass in the “on top of the litter” and “burrowing” interactions, possibly reflecting the abundance of this material in the ocean.

Our results showed that benthic octopuses that naturally shelter in mollusk shells interacted more commonly with marine litter when compared to species that use rocks and reef dens as shelter. Species that use seashells are usually found in sandy/muddy habitats where consolidated dens are rare – these animals therefore search for shells or other organic materials to use as shelter, but can choose litter instead.

Litter with small entrances can help protect small octopuses or juveniles from predators; meanwhile, larger species usually prefer larger shelters such as dens in rocks or reefs (Mather, 1994), and are rarely found sheltering in litter. The coconut octopus *A. marginatus*, registered in more than half of the evaluated images, typically occurs on sandy/muddy substrates in shallow waters using seashells, coconut halves or other marine litter to protect themselves (Jereb et al., 2013). This species can also carry the object by stilt-walking while searching for another shell in which to shelter (Finn et al., 2009). The predominance of litter use by species that naturally shelter in mollusk shells could be biased, since the main species observed (coconut octopus) occurs mainly along Asia, which is also where most images were taken. However, examples from other regions are also available. For instance, the recently described *Paroctopus ctchulu* seems to have found a solution for the lack of seashells in the environment at Ilha Grande (Rio de Janeiro state, Brazil): they shelter in the abundant beer cans discarded from tourist boats, including rusted ones (Leite et al., 2021). In this area, *P. ctchulu* has only been observed inside marine litter, and is commonly found walking around the dive boats after divers perform underwater cleanups and remove materials that animals were sheltered in.

Brown and transparent items were predominant in underwater images of octopus/litter interactions. There are several studies suggesting that cephalopods are colorblind (Gleadall and Shashar, 2004; Chung and Marshall, 2016), but it has been recently suggested that octopuses do have the ability to distinguish colors (Di Cosmo et al., 2018; Hanke and Kelber, 2020). Therefore, octopuses are able to discriminate objects

through polarization vision (Temple et al., 2021), and the predominance of these litter colors could be due to an active choice by octopuses, or simply be a reflection of the availability of these types of litter in the environment. In any case, translucency may influence litter use, and the higher occurrence of octopuses sheltering in dark glass beverage bottles/jars, incrustated items and metal cans could indicate selection due to their preference for shelters with light shading (Mather, 1982b), small entrances (Mather, 1982b) and large shelter size (Hartwick et al., 1984), characteristics that favor their protection. For instance, Anderson et al. (1999) reported that the octopus *O. rubescens* prefers to shelter in darker bottles, and we found three images of this species sheltering in metal and opaque glass. This shows that octopuses can adapt to anthropogenic change in the marine environment, but such adaptation could possibly lead to future problems if they become dependent on litter for survival. Chase and Verde (2011) performed laboratory experiments on shelter selection by *O. rubescens* and observed a significant preference for a dark/opaque bottle over a translucent one, although no preference was observed in the field. In addition, the common use of incrustated items could be due to their similarity to the natural environment, favoring the camouflage of these animals when compared to non-incrustated items. Further studies are necessary to evaluate this selection, as well as other factors involved in shelter choice.

Underwater images recorded competition for artificial resources between benthic octopuses and other organisms (such as other octopuses and hermit crabs). Since hermit crabs also use empty shells available in the substrate for protection (Fotheringham, 1973), this competition is another indication of the scarcity of seashells in marine ecosystems. This scarcity is likely due to removal by tourists and local populations, who commonly use shells to produce souvenirs; according to Kowalewski et al. (2014), this removal may result in habitat changes and decreases in the diversity/abundance of organisms that are dependent on shell availability. This could be also due to the general habitat loss of mollusks caused especially by habitat loss and anthropogenic disturbance such as urban pollution and coastal development (Peters et al., 2013). Additionally, laboratory studies indicate that ocean acidification caused by human activities negatively impacts shell production by juvenile and adult shelled mollusks (Cooley et al., 2012; Gazeau et al., 2013), which may also be related to this decrease in the availability of natural shelters in marine ecosystems. However, further studies are needed to confirm that bivalve populations are indeed decreasing.

The evaluated images showed octopuses using litter as shelter in all stages of their life cycle – paralarvae, juveniles, adults, and egg-laying females. Octopuses change shelters as they grow (Iribarne, 1990), and since most of the marine litter observed being used in the underwater images had smaller entrances (e.g. bottles, cans) than mollusk shells, octopuses likely have to seek new shelters frequently. Although this represents a natural behavior, the reduction in the entrance size increases search frequency, leading octopuses to be periodically exposed to predation during this search. Regarding female octopuses, laying their eggs in marine litter may affect their reproductive success, since the quality of the shelter (shape, overall condition) influences the area available for brooding, and can reduce the number of eggs deposited by females over time (Narvarte et al., 2013). Iribarne (1990) found that females brooding in unbroken bivalve/gastropod shells had significantly higher fertility than those brooding in broken shells or shelters made of artificial objects. The author speculates that this could be due to energy expenditure in holding together the parts of the shelter – energy that could be reallocated for the ventilation and care of the eggs – and to greater chances of predation and loss of eggs with the dislodgement of parts of the shelter.

The large number of underwater images evaluated in our work demonstrates how cephalopods are attractions in dives all over the world, especially in Asia, where there are thousands of famous diving spots (Lew, 2013) and where most cephalopod/litter interaction records were observed. This continent is also known to be highly polluted by

litter, and Meijer et al. (2021) report through a worldwide model that Asian rivers are the top emitters of litter to the ocean. However, since recreational diving is a relatively expensive activity, the difference in records between regions could also be related to the socio-economic conditions of the population or to the presence of highly touristic diving spots, and not necessarily with the frequency of occurrence of litter. For instance, there were no records for the African continent, where the consumption of single-use plastics is high and waste management is inefficient (Adam et al., 2020), and therefore high amounts of litter would be expected to enter the marine environment and be available for use by octopuses (Ryan, 2020).

Due to the natural behavior of shelter use by benthic octopuses, pots and traps are highly efficient in octopus fisheries (Mereu et al., 2018). The current literature recommends longline pots due to its high selectivity (exclusive for octopuses, which means no bycatch) and no physical impacts on the seafloor (Sauer et al., 2019). However, the basic gear consists of different materials, including ceramic, cement and more recently PVC tubes and plastics pots, which are the most concerning; the loss of these items increases the availability of shelters, but may have negative impacts on the environment, increasing the availability of litter. Octopus pots represented 94% of plastic litter collected on the seafloor of Morocco (Loulad et al., 2017), and 23% of plastics in coastal dunes of central-west Portugal (Andriolo et al., 2020). Once in the ocean, plastic pots may fragment and generate smaller plastic pieces, as already shown by Andrade (2015), and become highly bioavailable for different species (Ma et al., 2020). Thus, this type of fishery could have long-term consequences not only for octopus populations, but also for entire ecosystems, and we strongly recommend that pots be made of clay instead of plastic.

We observed, for the first time, deep-sea octopuses interacting with litter in the Mediterranean, where macro-litter occurs in high concentrations in deep-sea areas (Pierdomenico et al., 2019). The Mediterranean Sea is characterized by high levels of species endemism (Bianchi and Morri, 2000), but its coast is highly industrialized and urbanized, and the generated pollution is considered a major threat to biodiversity (Coll et al., 2010). Although poorly studied, it is already known that deep-sea zones have a diversity of habitats and provide numerous resources (Ramirez-Llodra et al., 2011), and new technologies (mainly ROVs) are showing how this environment is affected by natural and human impacts, especially marine litter (Melli et al., 2017; Pierdomenico et al., 2019). Angiolillo et al. (2015) observed that fishing gear was the main type (89%) of litter found in deep areas of the Mediterranean Sea, while other types of plastics were found only occasionally. Our results are in agreement with this, since three of our records were with fishing gear and the other two were with glass/ceramic fragments – which could have derived from octopus fisheries. Considering the importance of this ecosystem, further studies are necessary to clarify how marine litter is impacting its biodiversity, including cephalopods.

5. Conclusions and future directions

Underwater images from divers provided useful quantitative and qualitative data on the how octopuses interact with litter, and this is the first work to create a specific methodology for this type of data collection. Interaction records from divers provided important information, including affected octopus species and materials/types of litter chosen. Although this citizen science approach does not allow a more thorough evaluation of litter and interactions such as ingestion, it is a valuable and low-cost method of obtaining information from around the world to fill several knowledge gaps; an example of this is a recent paper that used digital media images to show the poorly studied negative impacts of ghost fishing gear on marine biodiversity in Brazil (Azevedo-Santos et al., 2021). Despite the possible positive impacts of using litter as shelter by octopuses, this interaction could have negative implications such as exposing animals to toxic compounds – such implications require additional investigation. It is possible that the negative impacts of litter

on octopuses is underestimated due to the lack of available data, and we therefore emphasize that the problem must be more thoroughly assessed. Based on the shortcomings discussed, the following priorities are suggested for future research:

1. Evaluation of shelter selection (in the field and in the laboratory) through experiments that assess which factors drive litter use by octopuses (e.g. opening size, volume, material, incrustations and/or translucency);
2. Clarification of the possible negative impacts of this choice and its long-term consequences to organisms and ecosystems;
3. Characterization of seafloor habitats, including amounts/types of available natural and artificial shelters, as well as the potential impacts of litter removal;
4. Investigation of litter interactions by different cephalopod groups (squid, cuttlefish and octopuses) in order to elucidate its actual occurrence and negative impacts.

In terms of citizen science initiatives and other actions, we suggest the following:

5. Application of this new methodology for other groups of organisms, in order to further improve observations;
6. Creation and dissemination of an underwater image database exclusively for cephalopod interactions with marine litter, in order to group these images;
7. Creation of citizen science campaigns specifically aimed towards divers and photographers;
8. Inclusion of protocols in underwater cleanups to check for animals living in marine litter before its discard.

Compared to litter present at the sea surface or beaches, as well as smaller litter size classes, studies on macro-litter at the seafloor are quite limited, especially due to the difficult logistics involved in sampling. Therefore, its impacts on different marine environments and their inhabitants are not fully understood, and further studies are needed to clarify this and generate solid information for creating prevention and management strategies. We emphasize that measures to prevent litter from reaching the ocean are essential to minimize its impacts: adequate waste management, public policies, international agreements, improvement of product materials/design, circularization of the plastic economy and public awareness are urgent measures to reduce litter impacts not only for cephalopods, but for all marine organisms and ecosystems.

CRediT authorship contribution statement

Tainah B.N. Freitas: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Tatiana S. Leite:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Bruna de Ramos:** Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Anna di Cosmo:** Investigation, Writing – review & editing. **Maira C. Proietti:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision, Project administration.

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.

Acknowledgements

This research was conducted within the scope of T. Barcat's master's degree in Biological Oceanography (Biological Oceanography Graduate Program of FURG), who received a scholarship from CAPES (Coordination for the Improvement of Higher Education Personnel). CAPES also provided resources through PROEX (Academic Excellence Program) and Projeto Ciências do Mar II - 23038.004807/2014-01. We are grateful to all divers, photographers, researchers, and all persons whose records contributed to the used image database. We also thank support from diving companies at Ilha Grande for all contributions on understanding the interactions between cephalopods and litter. CNPq (National Council for Technical and Scientific Development) provided a research scholarship to M. Proietti (Process 312470/2018-5).

References

- Adam, I., Walker, T.R., Bezerra, J.C., Clayton, A., 2020. Policies to reduce single-use plastic marine pollution in West Africa. *Mar. Policy* 116, 103928. <https://doi.org/10.1016/j.marpol.2020.103928>.
- Ambrose, R., 1982. Shelter utilization by the molluscan cephalopod *Octopus bimaculatus*. *Mar. Ecol. Prog. Ser.* 7, 67–73. <https://doi.org/10.3354/meps007067>.
- Anderson, R.C., Hughes, P.D., Mather, J.A., Steele, C.W., 1999. Determination of the diet of *Octopus rubescens* berry, 1953 (Cephalopoda: Octopodidae), through examination of its beer bottle dens in Puget Sound. *Malacologia* 41, 455–460.
- Andrade, L.C.A., 2015. Estratégias de Exploração e Comércio da Pesca Artesanal de Polvo. 129 f. Tese (Doutorado em Ecologia) - Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, 2015.
- Andriolo, U., Gonçalves, G., Bessa, F., Sobral, P., 2020. Mapping marine litter on coastal dunes with unmanned aerial systems: a showcase on the Atlantic Coast. *Sci. Total Environ.* 736, 139632. <https://doi.org/10.1016/j.scitotenv.2020.139632>.
- Angiolillo, M., di Lorenzo, B., Farcomeni, A., Bo, M., Bavestrello, G., Santangelo, G., Cau, Angelo, Mastascusa, V., Cau, Alessandro, Sacco, F., Canese, S., 2015. Distribution and assessment of marine debris in the deep Tyrrhenian Sea (NW Mediterranean Sea, Italy). *Mar. Pollut. Bull.* 92, 149–159. <https://doi.org/10.1016/j.marpolbul.2014.12.044>.
- Aronson, R.B., 1986. Life history and den ecology of *Octopus briareus* Robson in a marine lake. *J. Exp. Mar. Biol. Ecol.* 95, 37–56. [https://doi.org/10.1016/0022-0981\(86\)90086-9](https://doi.org/10.1016/0022-0981(86)90086-9).
- Avio, C.G., Gorbi, S., Regoli, F., 2017. Plastics and microplastics in the oceans: from emerging pollutants to emerged threat. *Mar. Environ. Res.* 128, 2–11. <https://doi.org/10.1016/j.marenvres.2016.05.012>.
- Azevedo-Santos, V.M., Marques, L.M., Teixeira, C.R., Giarrizzo, T., Barreto, R., Rodrigues-Filho, J.L., 2021. Digital media reveal negative impacts of ghost nets on Brazilian marine biodiversity. *Mar. Pollut. Bull.* 172, 112821. <https://doi.org/10.1016/j.marpolbul.2021.112821>.
- Barreiros, J.P., Luiz, O.J., 2009. Use of plastic debris as shelter by an unidentified species of hermit crab from the Maldives. *Mar. Biodivers. Rec.* 2, 1–2. <https://doi.org/10.1017/s1755267208000377>.
- Barreiros, J.P., Raykov, V.S., 2014. Lethal lesions and amputation caused by plastic debris and fishing gear on the loggerhead turtle *Caretta caretta* (Linnaeus, 1758). Three case reports from Terceira Island, Azores (NE Atlantic). *Mar. Pollut. Bull.* 86, 518–522. <https://doi.org/10.1016/j.marpolbul.2014.07.020>.
- Barros, F., Santos, D., Reis, A., Martins, A., Dodonov, P., Anchieta, J., Nunes, C.C., 2020. Choosing trash instead of nature: sea urchin covering behavior. *Mar. Pollut. Bull.* 155, 111188. <https://doi.org/10.1016/j.marpolbul.2020.111188>.
- Bianchi, C.N., Morri, C., 2000. Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Mar. Pollut. Bull.* 40, 367–376. [https://doi.org/10.1016/S0025-326X\(00\)00027-8](https://doi.org/10.1016/S0025-326X(00)00027-8).
- Brandão, M.L., Braga, K.M., Luque, J.L., 2011. Marine debris ingestion by magellanic penguins, *Spheniscus magellanicus* (Aves: Sphenisciformes), from the Brazilian coastal zone. *Mar. Pollut. Bull.* 62, 2246–2249. <https://doi.org/10.1016/j.marpolbul.2011.07.016>.
- Canals, M., Pham, C.K., Bergmann, M., Gutow, L., Hanke, G., van Sebille, E., Angiolillo, M., Buhl-Mortensen, L., Cau, A., Ioakeimidis, C., Kammann, U., Lundsten, L., Papatheodorou, G., Purser, A., Sanchez-Vidal, A., Schulz, M., Vinci, M., Chiba, S., Galgani, F., Langenkämper, D., Möller, T., Nattkemper, T.W., Ruiz, M., Suikkanen, S., Woodall, L., Fakiris, E., Molina Jack, M.E., Giorgetti, A., 2021. The quest for seafloor macrolitter: a critical review of background knowledge, current methods and future prospects. *Environ. Res. Lett.* 16. <https://doi.org/10.1088/1748-9326/abc6d4>.
- de Carvalho-Souza, G.F., Llope, M., Tinoco, M.S., Medeiros, D.V., Maia-Nogueira, R., Sampaio, C.L.S., 2018. Marine litter disrupts ecological processes in reef systems. *Mar. Pollut. Bull.* 133, 464–471. <https://doi.org/10.1016/j.marpolbul.2018.05.049>.
- Ceccaroni, L., Bowser, A., Brenton, P., 2017. Civic education and citizen science: definitions, categories, knowledge representation. *Anal. Role Citiz. Sci. Mod. Res.* 1–23. <https://doi.org/10.4018/978-1-5225-0962-2.ch001>.
- Chase, E.R., Verde, E.A., 2011. In: Population Density and Choice of Den and Food Made by *Octopus rubescens* Collected From Admiralty Bay, Washington, in July 2011, pp. 110–116.

- Sauer, W., Gleadall, I., Downey-Breedt, N., Doubleday, Z., Gillespie, G., Haimovici, M., Ib'anez, C., Katugin, O., Leporati, S., Lipinski, M., Markaida, U., Ramos, J., Rosa, R., Villanueva, R., Arguelles, J., Briceno, F., Carrasco, S., Che, L., Chen, C., Cisneros, R., Conners, E., Crespi-Abril, A., Kulik, V., Drobyazin, E., Emery, T., FernandezAlvarez, F., Furuya, H., Gonzalez, L., Gough, C., Krishnan, P., Kumar, B., Leite, T., Lu, C., Mohamed, K., Nabhitabhata, J., Noro, K., Petchkamnerd, J., Putra, D., Rocliffe, S., Sajikumar, K., Sakaguchi, H., Samuel, D., Sasikumar, G., Wada, T., Zheng, X., Tian, Y., Pang, Y., Yamrungrueng, A., Pecl, G., 2019. World Octopus fisheries. *Rev. Fish. Sci. Aquac.* <https://doi.org/10.1080/23308249.2019.1680603>.
- Smith, S.D.A., 2012. Marine debris: a proximate threat to marine sustainability in Bootless Bay, Papua New Guinea. *Mar. Pollut. Bull.* 64, 1880–1883. <https://doi.org/10.1016/j.marpolbul.2012.06.013>.
- Souto Alves, M., Aparecida da Silva, M., Melo Júnior, M., Nogueira Paranaguá, M., de Lyra Pinto, S., 2006. *Zooartesanato comercializado em Recife, Pernambuco, Brasil*. *Rev. Bras. Zootecias* 8, 99–109.
- Temple, S.E., How, M.J., Powell, S.B., Gruev, V., Marshall, N.J., Roberts, N.W., 2021. Thresholds of polarization vision in octopuses. *J. Exp. Biol.* 224, 1–7. <https://doi.org/10.1242/jeb.240812>.
- Uhrin, A.V., Schellinger, J., 2011. Marine debris impacts to a tidal fringing-marsh in North Carolina. *Mar. Pollut. Bull.* 62, 2605–2610. <https://doi.org/10.1016/j.marpolbul.2011.10.006>.
- Vidal, E.A.G., Villanueva, R., Andrade, J.P., Gleadall, I.G., Iglesias, J., Koueta, N., Rosas, C., Segawa, S., Grasse, B., Franco-Santos, R.M., Albertin, C.B., Caamal-Monsreal, C., Chimal, M.E., Edsinger-Gonzales, E., Gallardo, P., Le Pabic, C., Pascual, C., Roumbekakis, K., Wood, J., 2014. Cephalopod culture: current status of main biological models and research priorities. *Adv. Mar. Biol.* <https://doi.org/10.1016/B978-0-12-800287-2.00001-9>.
- Voight, J.R., 1992. Movement, injuries and growth of members of a natural population of the Pacific pygmy octopus, *Octopus digueti*. *J. Zool.* 228, 247–264. <https://doi.org/10.1111/j.1469-7998.1992.tb04606.x>.
- Wegner, N.C., Cartamil, D.P., 2012. Effects of prolonged entanglement in discarded fishing gear with substantive biofouling on the health and behavior of an adult shortfin mako shark, *Isurus oxyrinchus*. *Mar. Pollut. Bull.* 64, 391–394. <https://doi.org/10.1016/j.marpolbul.2011.11.017>.