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INGESTÃO DE LIXO PLÁSTICO MARINHO POR TARTARUGAS MARINHAS NO SUL DO BRASIL: ABUNDÂNCIA, CARACTERÍSTICAS E SELETIVIDADE

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“It has been suggested that sea turtles eat debris because it resembles their natural prey or perhaps because epizoic or epiphytic growth on the debris has attracted the turtle. Before man began discarding his nonbiodegradable wastes into the oceans, sea turtles did not have to differentiate between what was edible and what was not, because essentially everything was edible (Plotkin & Amos, 1990)”

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RESUMO

Os impactos causados pelo lixo plástico marinho (LPM) têm sido comumente reportados devido às interações negativas com a biota marinha. A ingestão é uma destas interações e já foi observada para diversas espécies incluindo tartarugas. Na costa brasileira ocorrem cinco espécies de tartarugas marinhas: a tartaruga-verde *Chelonia mydas*, a tartaruga-cabeçuda *Caretta caretta*, a tartaruga-de-pente *Eretmochelys imbricata*, a tartaruga-oliva *Lepidochelys olivacea* e a tartaruga-de-couro *Dermochelys coriacea*. Todas estas espécies estão ameaçadas de extinção e identificar os fatores que levam à sua interação com o LPM é uma prioridade para a conservação. Neste estudo, quantificamos e caracterizamos o LPM ingerido pelas cinco espécies de tartarugas marinhas que ocorrem no litoral sul do Rio Grande do Sul, Brasil, de acordo com a biologia e ecologia das espécies e identificamos as características do LPM ingerido, e avaliamos esta ingestão pela tartaruga-verde através do tempo. Foram coletados 86 tratos gastrointestinais de tartarugas marinhas provenientes de encalhes e capturas incidentais na pesca entre 2013 e 2017. O LPM encontrado foi quantificado e caracterizado, e foram avaliadas diferenças entre espécies, tamanho, habitat ocupado, estratégia alimentar e preferência por tipo e cor. A variação temporal da ingestão pela tartaruga-verde foi avaliada a partir de dados de 1997 a 2017. O LPM foi encontrado em 49 dos 86 indivíduos (57%) e em todas as espécies, com a tartaruga-verde apresentando maior ingestão (81%). Os plásticos representaram 97% dos itens, sendo as embalagens, linhas de pesca e fragmentos rígidos os mais ingeridos. Alguns itens não plásticos foram encontrados em baixa frequência (3%), sendo comuns os balões de borracha. A tartaruga-verde está sob contínua e elevada ameaça do LPM na região de estudo ao menos desde 1997, data do primeiro estudo abordando o tema. Os indivíduos de habito alimentar onívoro (80%) apresentaram ingestão de LPM superior aos carnívoros (25%), enquanto que indivíduos dos habitats nerítico e oceânico apresentaram ingestão semelhante (58% e 50%, respectivamente). Em tartarugas-verdes a quantidade de LPM ingerida não teve correlação significativa com o tamanho do indivíduo coletado; já para as tartarugas-cabeçudas foi observada correlação significativa negativa. Para a tartaruga-verde o modelo linear generalizado (MLG) demonstrou haver interação entre o tipo e cor dos itens ingeridos. Com relação ao Índice de Importância Relativa Presa-específica (%PSIRI) os fragmentos flexíveis transparentes (29%), flexíveis brancos (12%) e rígidos brancos (12%) foram os mais importantes. Para a tartaruga-cabeçuda o MLG não demonstrou interação entre tipo e cor do LPM. Os fragmentos rígidos (PSIRI = 30%), fragmentos flexíveis (18%), isopor/espuma (18%), e as cores branca (40%) e preto/marrom (29%) apresentaram os maiores %PSIRI. Os resultados obtidos auxiliam no entendimento da ingestão de LPM pelas diferentes espécies de tartarugas marinhas, e fornecem informações de base para definição de políticas de prevenção e mitigação para este problema global.

Palavras-chave: poluição plástica marinha, tartarugas marinhas, impactos, Atlântico Sul Ocidental, análise temporal, índice de seletividade.

ABSTRACT

The impacts caused by plastic marine litter (PML) have been commonly reported due to negative interactions with marine biota. One of these interactions is ingestion, which has been observed for numerous species including turtles. At the Brazilian coast five sea turtle species occur: the green *Chelonia mydas*, loggerhead *Caretta caretta*, hawksbill *Eretmochelys imbricate*, olive ridley *Lepidochelys olivacea*, and leatherback turtle *Dermochelys coriacea*. All of these species are considered threatened and identifying the factors that lead to their interaction with PML is a priority. In this work, we quantified and characterized PML ingested by the five sea turtle species that occur in the coast of South of Rio Grande do Sul state, Brazil, according to the biology and ecology of species and characteristics of PML, and evaluated the ingestion of such litter by green turtles over time. Gastrointestinal tracts of sea turtles (n = 86) from beach strandings and bycatch were collected from 2013 to 2017. When found, PML was quantified and characterized, and differences between species, size, occupied habitat, feeding strategy and preference for type and color were evaluated. Temporal variation in ingestion by green turtles was analysed using data from 1997 to 2017. PML was found in 49 of the 86 individuals (57%), in all species, with green turtles presenting highest ingestion rate (81%). Plastics represented 97% of items, with packaging, fishing lines and hard fragments the most ingested. Some non-plastic items were also found with less frequency (3%), with rubber balloons being the most common. Green turtles are under continuous and high threat due to PML at the region, at least since 1997. Individuals classified with feeding strategy omnivorous presented PML ingestion higher than those classified as carnivorous (80% and 25%, respectively), while neritic and oceanic animals habitats presented similar ingestion (58% and 50%, respectively). Turtle size and amounts of ingested PML were not significantly correlated for green turtles, but were significantly negatively correlated in loggerheads. General linearized models (GLM) showed that for the green turtle there was interaction between the type and color of ingested items. The Prey-Specific Index of Relative Importance (%PSIRI) show that flexible transparent (29%), flexible white (12%) and hard white fragments (12%) were the most important. For the loggerhead the GLM demonstrated that there was no interaction between type and color. Hard fragments (PSIRI = 30%), flexible fragments(18%), closed-cell extruded polystyrene foam (XPS)/foam (18%), white (40%) and black/brown colors (29%) presented the highest %PSIRI. These results help us better understand PML ingestion by different sea turtle species and provide baseline information for the definition of prevention and mitigation strategies for this global issue.

Keywords: marine plastic pollution, marine turtles, Western South Atlantic, impacts, temporal analysis, selectivity index.

1. INTRODUÇÃO

Uma grande ameaça aos oceanos e à vida marinha é a poluição ocasionada pelo lixo plástico marinho (LPM), o que representa um impacto amplamente distribuído e duradouro (Derraik 2002, Barnes et al. 2009, Gregory 2009). O LPM é constituído de materiais sólidos sintéticos de origem humana, que entram nos ambientes marinhos e costeiros (Coe & Rogers 2000) e compõem um dos problemas de poluição mais comuns em águas interiores e nos oceanos do mundo (Sheavly & Register 2007). O LPM acumula-se em praticamente todas as regiões, dos polos ao equador, da superfície dos oceanos ao fundo marinho e até mesmo em áreas remotas, como ilhas oceânicas (UNEP

2009, Barnes et al. 2009, Cózar et al. 2014). A origem do LPM pode ser terrestre (principal fonte) proveniente de resíduos domésticos, industriais e de turismo costeiro, que são carreados diretamente ou por sistemas de esgoto e fluviais, escoamento superficial e por ventos para as praias e oceanos; ou também pode se originar diretamente no ambiente marinho, derivado de atividades pesqueiras e descartes de plataformas de petróleo, embarcações comerciais, militares e até mesmo de pesquisa (Ivar do Sul & Costa 2007, Sheavly & Register 2007, Jambeck et al. 2015, Lebreton et al. 2017). As regiões de acúmulo de LPM variam amplamente e são influenciadas por fatores como a proximidade com áreas urbanizadas, atividades marítimas e as condições oceanográficas dominantes (Galgani et al. 2015).

Os impactos causados pelo LPM estiveram diretamente ligados ao desenvolvimento dos polímeros sintéticos (plásticos) (UNEP 2016), que constituem aproximadamente 90% do LPM (Derraik 2002, Thompson et al. 2009, Ryan 2014). A produção de plásticos tem crescido exponencialmente desde o início da década de 1930 (Thompson et al. 2009) e entre 2000 e 2016, aumentou de 200 para 335 milhões de toneladas por ano (Plastics Europe 2017). Com base em Jambeck et al. (2015), é estimado que entre 5,7 e 15,4 milhões de toneladas métricas de plásticos tenha entrado nos oceanos em 2016, devido à inadequada destinação e gestão destes resíduos. O aumento da produção, aliado ao descarte inadequado, faz com que o LPM esteja entrando nos oceanos em crescente quantidade, aumentando a sua disponibilidade e resultando em maiores probabilidades de interações com a vida marinha.

As interações entre a biota marinha e o LPM ocorrem de diversas formas como através da ingestão, emaranhamento, transferência de contaminantes via cadeia trófica, uso como substrato por espécies invasoras e sufocamento dos organismos bentônicos (Derraik 2002, Gregory 2009). Destas interações, a ingestão é um dos impactos mais reportados, com registros de ao menos 395 espécies de diversos grupos animais sendo afetadas de forma negativa (Gall & Thompson 2015). A ingestão de LPM pode resultar na morte dos animais ou gerar efeitos sub-letais no desenvolvimento de indivíduos de espécies marinhas (Oehlmann et al. 2009). Dentre estes efeitos sub-letais, citam-se lesões internas, bloqueio no trato gastrointestinal, enfraquecimento e alterações na flutuabilidade (Gregory 2009, Oehlmann et al. 2009). Adicionalmente, os impactos do LPM podem incluir efeitos na cadeia trófica, uma vez que os aditivos e poluentes associados aos plásticos podem ser bioacumulados e biomagnificados até chegarem aos consumidores de nível trófico mais elevado (Reisser et al. 2014).

Os primeiros registros de ingestão de LPM por tartarugas marinhas foram relatados na década de 1980 (Balazs 1984, Carr 1987), e desde então este impacto negativo tem sido comumente reportado (Ivar do Sul & Costa 2007, Schuyler et al. 2014a, Nelms et al. 2015). Em uma análise global sobre o tema, Schuyler et al. (2014a) verificaram que 96,8% (n = 30 artigos) dos estudos reportaram a ingestão de lixo por tartarugas marinhas, com ocorrência nas sete espécies existentes (Kühn et al. 2015), o que de certa forma, representa uma séria ameaça para estes animais mundialmente considerados em perigo de extinção. Segundo a Lista Vermelha de Espécies Ameaçadas da União Internacional para a Conservação da Natureza (IUCN 2018), as cinco espécies de tartarugas marinhas que ocorrem na costa brasileira são classificadas quanto ao grau de ameaça como: vulnerável (tartaruga-cabeçuda - *Caretta caretta*, tartaruga-de-couro - *Dermochelys coriacea* e tartaruga-oliva - *Lepidochelys olivacea*), em perigo (tartaruga-verde - *Chelonia mydas*) e criticamente em perigo (tartaruga-de-pente - *Eretmochelys imbricata*). Já o status de ameaça conforme a Lista Brasileira de Espécies Ameaçadas (MMA 2014) aponta a tartaruga-verde como vulnerável, a tartaruga-cabeçuda e tartaruga-oliva como em perigo e a tartaruga-de-couro e tartaruga-de-pente como criticamente em perigo.

A ingestão de lixo pode ocorrer de diferentes formas no ambiente marinho: diretamente, quando o animal identifica incorretamente o material e o seleciona ativamente pela similaridade com suas presas (e.g. sacolas plásticas e balões confundidos com medusas) (Schuyler et al. 2012, Hoarau et al. 2014, Schuyler et al. 2014b); e indiretamente, quando o lixo é ingerido acidentalmente durante o consumo de suas presas (e.g. pastagem sobre gramíneas e algas marinhas pela tartaruga-verde) ou ingestão de cracas ou outros organismos aderidos ao lixo (Di Beneditto & Awabdi 2014). Considerando que as tartarugas marinhas podem ocupar diferentes habitats e adotar distintos hábitos alimentares ao longo do seu ciclo de vida, estes fatores podem influenciar a ingestão devido à disponibilidade de LPM em diferentes ambientes e o modo de forrageio dos animais (Schuyler et al. 2014a).

Após o nascimento, a maioria das espécies de tartarugas permanece entre 1 e 13 anos nos sistemas de correntes superficiais oceânicas (Boulon 1994, Bjorndal et al.

1997, Lenz et al. 2016, 2017), estando mais susceptíveis à ingestão de fragmentos plásticos flutuantes do que durante o período de alimentação bentônica (Schuyler et al. 2012). Após crescerem, algumas espécies podem permanecer no ambiente oceânico, ou passarem a ocupar o ambiente nerítico (Bolten 2003), onde podem interagir com o LPM devido à proximidade de fontes continentais de resíduos sólidos (Schuyler et al. 2012). Em relação à estratégia alimentar, as tartarugas marinhas apresentam diferenças interespecíficas, assim como mudanças intraespecíficas de acordo com seu estágio de vida, onde filhotes tendem a ser mais generalistas, se tornando mais especialistas nos estágios juvenil e adulto, o que pode resultar em uma menor probabilidade de ingestão de plásticos (Schuyler et al. 2014a).

As cinco espécies de tartarugas marinhas que ocorrem na costa brasileira são encontradas em diferentes graus de frequência e abundância, no litoral do Rio Grande do Sul (RS). A tartaruga-verde ocorre na região principalmente quando juvenil e neste estágio sua alimentação é composta por algas, gramíneas, moluscos, peixes, anêmonas e medusas (Bjorndal et al. 1997, Bugoni et al. 2003, Carman et al. 2014). A tartaruga-de-pente alimenta-se principalmente de organismos bentônicos sésseis, como zoantídeos e esponjas, mas também pode ingerir algas, celenterados e crustáceos (Grossman et al. 2006, Proietti et al. 2012). A tartaruga-cabeçuda, quando no ambiente oceânico, alimenta-se principalmente de organismos gelatinosos como salpas e pirossomos, e no ambiente nerítico de invertebrados bentônicos (crustáceos e moluscos) e peixes (Barros 2010, Di Beneditto et al. 2015). A alimentação da tartaruga-oliva é composta primariamente por peixes, crustáceos e moluscos (Colman et al. 2014, Di Beneditto et al. 2015), podendo ingerir organismos gelatinosos no ambiente oceânico (Di Beneditto et al. 2015). Já a tartaruga-de-couro tem a sua alimentação composta por organismos gelatinosos como cnidários, pirossomos e ctenóforos (Saba 2013).

O litoral do RS é uma importante área de alimentação para a tartaruga-verde, a tartaruga-cabeçuda e a tartaruga-de-couro, e apresenta a ocorrência esporádica da tartaruga-oliva e da tartaruga-de-pente (Monteiro et al. 2016). Encalhes no sul do Brasil ocorrem ao longo de todo o ano, com maior frequência nos meses de outubro a março (primavera-verão austral) (Monteiro et al. 2016). Nesta região, ao longo dos últimos 10 anos, houve um aumento no número de encalhes de tartaruga-cabeçuda e tartaruga-verde (de dezenas para centenas de encalhes ao ano), assim como um aumento dos registros de tartaruga-oliva e tartaruga-de-pente (Monteiro et al. 2016). Para a tartaruga-verde, já se observou a ingestão de LPM em mais de 60% dos indivíduos provenientes de encalhes (Bugoni et al. 2001, Tourinho et al. 2010, Colferai et al. 2017), enquanto que para a tartaruga-cabeçuda foi observada ingestão de LPM em mais de 90% das tartarugas provenientes de captura incidental oceânica, porém com baixa ocorrência nos indivíduos provenientes de encalhes (Barros 2010). Para a tartaruga-de-couro há registro de ingestão de LPM, com duas ocorrências em cinco indivíduos analisados (Pinedo et al. 1996, Bugoni et al. 2001).

Entender o grau de vulnerabilidade das espécies marinhas ao lixo presente nos oceanos é fundamental para auxiliar na elaboração de medidas de prevenção e mitigação apropriadas para estas espécies-bandeira da conservação marinha (Eckert & Hemphill 2005, Nelms et al. 2015). Embora os impactos físicos da ingestão de LPM pelas tartarugas marinhas sejam bem definidos, os fatores que levam a esta interação ainda são pouco conhecidos. A compreensão das formas de ingestão de plástico em diferentes espécies de tartarugas marinhas permitirá identificar similaridades quanto à ingestão e às características dos itens ingeridos por espécies que possuem alimentação e uso de habitat distintos. A ingestão de LPM pelas tartarugas marinhas é difícil de ser avaliada, principalmente devido à sua distribuição relativamente contínua e realizarem grandes migrações; no entanto, a avaliação da ingestão é identificada como uma prioridade de pesquisa para estes animais (Vegter et al. 2014).

1.1 Hipóteses

Neste trabalho hipotetizou-se que (i) devido à grande disponibilidade de LPM nos oceanos, todas as espécies de tartarugas marinhas apresentam LPM em seus tratos gastrointestinais; (ii) a crescente entrada de itens plásticos nos oceanos resulta em um aumento na ocorrência da ingestão ao longo do tempo; (iii) as tartarugas marinhas de alimentação generalista, as que ocupam o habitat oceânico e as que possuem menor tamanho apresentam uma alta quantidade e frequência de lixo ingerido; (iv) os itens de lixo marinho mais semelhantes às presas das tartarugas marinhas são mais consumidos.

1.2 Objetivos

O objetivo geral do presente estudo foi avaliar a quantidade e variedade do LPM ingerido por tartarugas marinhas que ocorrem no sul do Rio Grande do Sul, assim como possíveis variações temporais na sua ingestão pela tartaruga-verde e variações entre habitats pela tartaruga-cabeçuda. Os objetivos específicos foram: 1) caracterizar e quantificar o LPM ingerido pelas tartarugas marinhas; 2) verificar a frequência de ocorrência de LPM nos tratos digestórios; 3) comparar no tempo (1997-2017) a ingestão de LPM pela tartaruga-verde; 4) verificar a influência da espécie, habitat ocupado, estratégia alimentar e tamanho da tartaruga na quantidade do LPM ingeridos; e 5) avaliar se as tartarugas selecionam o LPM de acordo com determinadas características (p.e. tipo, cor, polímero).

2. MATERIAL E MÉTODOS

2.1. Área de estudo

As amostras de trato gastrointestinal (TGI) deste estudo foram coletadas de tartarugas marinhas que encalharam no litoral sul do Rio Grande do Sul (RS), em uma extensão de praia de aproximadamente 350 km, entre a Lagoa do Peixe (31°20'S; 51°05'W) e o Arroio Chuí (33°45'S; 53°22'W). Adicionalmente, foram coletadas amostras de TGI de tartarugas provenientes de captura incidental (*bycatch*) de embarcações pesqueiras de arrasto de parelha, atuantes na plataforma continental interna adjacente à desembocadura da Lagoa dos Patos (Fig. 1).

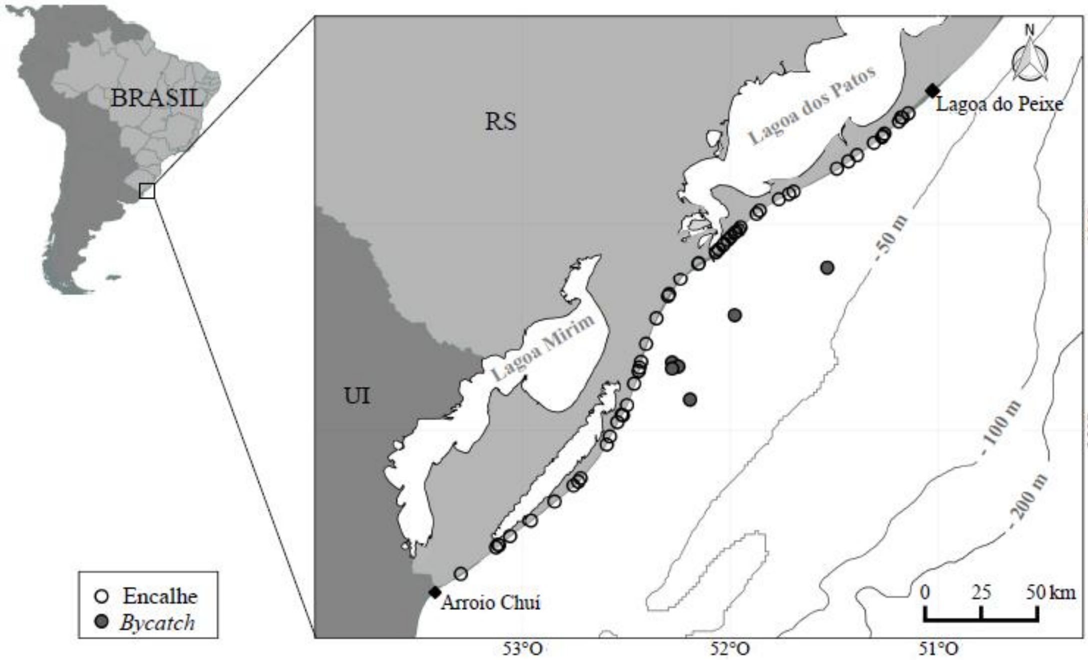

Fig.1. Área de estudo com locais de coletas (círculos) ao longo da costa do sul do Rio Grande do Sul. RS = Rio Grande do Sul, Brasil; UI = Uruguai.

O litoral do RS apresenta uma orientação nordeste-sudoeste, com praias arenosas retilíneas e contínuas, predominantemente dominadas por ondas (Calliari et al. 2005). No Atlântico Sul Ocidental, a Corrente do Brasil flui para o sul ao longo da fronteira oeste do giro subtropical e a Corrente das Malvinas segue para o norte ao longo da borda oeste da Bacia Argentina encontrando-se aproximadamente em 38°S, criando a Confluência Brasil-Malvinas (Oliveira et al. 2009). Parte das águas formadas por esta interação flui em direção ao norte sobre a plataforma continental do Uruguai e sul do Brasil, misturando-se com as águas do Rio da Prata e em menor escala, da Lagoa dos Patos, formando a Frente Subtropical de Plataforma (STSF). Esta frente é uma estrutura termohalina complexa que se estende mais ao norte durante o inverno e mais ao sul durante o verão austral (Piola et al. 2000). A interação dinâmica destas diferentes massas de água cria uma região com águas de alto teor de nutrientes, elevada produção primária e secundária, e que sustentam importantes atividades pesqueiras na região (Muelbert et al. 2008), tornando-a um importante local de alimentação e desenvolvimento para inúmeras espécies marinhas, incluindo as tartarugas.

2.2. Amostragem e processamento das amostras

Foram coletados 86 TGIs de tartarugas marinhas, sendo 80 TGIs provenientes de monitoramentos de praia realizados no período de maio de 2013 até novembro de 2017, pelo Núcleo de Educação e Monitoramento Ambiental (NEMA). Os outros seis indivíduos foram capturados incidentalmente (*bycatch*) na pescaria de arrasto de parelha que atua sobre a plataforma continental interna da área de estudo e coletados por observadores de bordo do NEMA entre 2015 e 2017. Para cada indivíduo foi registrado o comprimento curvilíneo da carapaça (CCC) em centímetros, tomado a partir do ponto anterior médio do escudo nucal até o entalhe posterior médio dos escudos supracaudais; o CCC para as tartarugas-de-couro foi medido do início da quilha nucal (borda anterior da carapaça na linha central) até a ponta posterior do pedúnculo caudal (Bolten 1999). Informações referentes ao ponto de encalhe e captura (latitude e longitude) e a data de cada observação foram registradas. As tartarugas encalhadas mortas e as provenientes das embarcações pesqueiras foram necropsiadas em campo, com cada TGI sendo congelado para posterior análise. Tartarugas encalhadas vivas foram encaminhadas ao Centro de Recuperação de Animais Marinhos (CRAM-FURG) para reabilitação, e quando vieram a óbito foram submetidas ao mesmo procedimento de necropsia.

Os TGIs foram retirados mediante incisão desde o esôfago até a porção final do intestino grosso, para posterior triagem em laboratório. O TGI (esôfago, estômago e intestino) de cada indivíduo foi avaliada e o LPM foi separado do material orgânico (conteúdo alimentar) (Fig.2), e lavados em peneira com malha de 1 mm de diâmetro. Para quantificar a proporção de LPM em relação ao material orgânico, ambos foram pesados (massa úmida) em balança com precisão de 0,1 g. A presença de obstruções no TGI foi registrada para posterior avaliação de possíveis danos causados pelo LPM. Considerou-se como obstrução do TGI quando se observou algum fragmento preso à parede do TGI ou havia formação de fecalomas (material fecal endurecido). Após a lavagem, os itens de LPM (Fig. 2) foram secos em estufa a 60°C e cada item foi quantificado, pesado (g), calculado o volume (comprimento × largura × altura - cm^3) e caracterizado de acordo com seu material constituinte, conforme o guia da UNEP (2009). Os itens foram caracterizados em termos de material (plástico, borracha, madeira, papel e tecido, vidro, metal), tipo (característica do item, e.g. fragmento, copo, espuma, sacola), cor (branco, transparente, preto, marrom, azul, verde, cinza, amarelo, vermelho, rosa, laranja, colorido) e flexibilidade (rígido ou flexível).

Fig. 2. Lixo plástico marinho (LPM) ingerido pelas tartarugas marinhas: (a) conteúdo de trato gastrointestinal de tartaruga-verde *Chelonia mydas* com fragmentos de LPM; (b) conteúdo de trato gastrointestinal de tartaruga-cabeçuda *Caretta caretta* com fragmento de calçado; (c) e (d) fragmentos flexíveis rígidos ingeridos por um indivíduo de tartaruga-verde *Chelonia mydas*.

Para a determinação dos polímeros plásticos ingeridos, foram selecionadas aleatoriamente 128 amostras (fragmentos) de LPM ingeridas pelas espécies e de cada uma foi recortado um pedaço de aproximadamente 3 × 3 mm. As amostras foram mantidas continuamente em estufas a 40°C para secagem por um período entre três a sete meses (tempo necessário para a secagem de cada amostra depende do tipo de polímero). A composição dos polímeros foi identificada através da análise de Espectroscopia Vibracional no Infravermelho por Transformada de Fourier (FTIR), seguindo a normativa ATSM SP E1252 − 98, utilizando o equipamento Prestige 21, com módulo de refletância difusa, 24 varreduras e resolução de 4 cm^{-1}. A análise de picos foi efetuada considerando apenas sinais acima de 50% de intensidade e considerando o pico mais intenso. Os espectros gerados dos fragmentos amostrados foram comparados com os espectros conhecidos de polímeros plásticos (Silverstein et al. 2007), permitindo identificar os polímeros e seus sinais de degradação. Estas análises foram realizadas na Escola de Química e Alimentos (EQA-FURG).

2.3. Análise de dados

2.3.1. Parâmetros de ingestão do LPM

A ingestão de LPM pelas tartarugas marinhas foi avaliada através dos seguintes parâmetros: número de itens (N), massa em gramas (M) e volume em cm^3 (V) de itens encontrados por espécie, por indivíduo e parte do TGI. Foi calculada a Frequência de Ocorrência relativa (FO%), referente ao percentual da ocorrência de LPM em relação ao número total de conteúdos analisados por espécie e parte do TGI.

2.3.2. Estágios do ciclo de vida

Os indivíduos coletados foram classificados conforme seu estágio do ciclo de vida, em juvenis ou adultos (Tabela 1). Esta classificação foi feita com base nos CCCs mínimos observados nas áreas de desova mais importantes do litoral brasileiro para cada espécie como sugerido por Monteiro et al. (2016). Indivíduos menores que estes tamanhos mínimos pré-estipulados foram considerados juvenis e maiores adultos.

Tabela 1. Comprimento curvilíneo da carapaça (CCC) e estágio de vida das tartarugas marinhas avaliadas. DP = desvio padrão.

Espécie	Origem da coleta	CCC mín – máx (cm) (média ± DP)	CCC mínimo na desova (cm)	Estágio de vida
Chelonia mydas (n = 48)	Encalhe	28 - 60 (39 ± 7)	90[a]	juvenis
Caretta caretta (n = 24)	Encalhe e *bycatch*	40 - 107 (73,5 ± 15)	83[b]	19 juvenis, 5 adultos
Lepidochelys olivacea (n = 8)	Encalhe e *bycatch*	62 - 72 (67,9 ± 3,5)	63[c]	1 juvenil, 7 adultos
Dermochelys coriacea (n = 4)	Encalhe	107 – 170 (141 ± 22)	139[d]	2 juvenis, 2 adultos
Eretmochelys imbricata (n = 2)	Encalhe	33 – 37 (34,8 ± 2,5)	86[e]	juvenis

[a]Almeida et al. 2011,[b]Baptistotte et al. 2003,[c]Silva et al. 2007,[d]Thomé et al. 2007,[e]Marcovaldi et al. 1999

2.3.3 Comparação temporal da ingestão de LPM – tartaruga-verde (Chelonia mydas)

A variabilidade temporal na ingestão de LPM foi avaliada por meio de uma comparação com os dados de estudos pretéritos realizados na mesma região. Os dados brutos da ingestão de LPM pela tartaruga-verde foram fornecidos pelos autores destes estudos, e compreendem os seguintes períodos: 1997 (Bugoni et al. 2001), 2006-2007 (Tourinho et al. 2010), 2010 (Ruzzene 2011), 2011-2014 (Colferai et al. 2017) e 2013-2017 (presente estudo). Não há dados entre 1997-2006 e entre 2008-2009 devido à ausência de estudos do tema nestes períodos.

Inicialmente, realizou-se uma análise exploratória dos dados pretéritos, os quais foram reorganizados e padronizados. Desta forma, foi possível identificar as variáveis em comum para os cinco períodos: ano de coleta, presença/ausência de LPM nos TGIs e massa (g) média de materiais sintéticos, por indivíduo. Para analisar a variação na ingestão de LPM ao longo do tempo, foi calculada a FO% da ingestão e a massa de lixo ingerido por indivíduo. Em 1997 (Bugoni et al. 2001) somente o LPM encontrado no esôfago e estômago dos indivíduos foi avaliado. Por isso foi feita uma correção para todo o TGI, baseado nos dados de Colferai et al. (2017) e do presente estudo, para: massa, calculado através da porcentagem que o esôfago e estômago representavam do LPM total e extrapolando para todo o TGI; e FO%, através do aumento da FO% quando calculado para o esôfago e estômago em relação ao total do TGI, considerando os TGIs em que havia somente LPM no intestino. Os dados não apresentaram distribuição normal, conforme teste de Shapiro-Wilk (Royston, 1992) e para comparar as médias de massa de LPM entre os anos foi realizado um teste de Kruskal-Wallis. Em seguida, para comparações par-a-par entre as médias de massa foi realizado o teste não-paramétrico de Dunn (Dunn 1964).

2.3.4. Habitat e estratégia alimentar

Para avaliar a relação entre a ingestão de LPM e o habitat ocupado, os indivíduos foram classificados em dois grupos: neríticos e oceânicos. Para a tartaruga-verde e tartaruga-cabeçuda, o habitat foi classificado com base no tamanho de primeiro recrutamento para o ambiente nerítico, que para o Rio Grande do Sul é de 30 cm para a tartaruga-verde (Lenz et al. 2016) e 55 cm para a tartaruga-cabeçuda (Lenz et al. 2017). Os indivíduos da tartaruga-de-pente foram classificados com base no tamanho de primeiro recrutamento para o Atlântico (Meylan 1988) e Porto Rico (Diez & van Dam 2002), sendo 20 cm o tamanho mínimo para ambas as localidades. As populações de tartaruga-oliva do Atlântico Oeste recrutam para a região nerítica após uma parte de seu desenvolvimento no ambiente oceânico (Reichart 1993, Bolten 2003, Silva et al. 2011). Deste modo, os indivíduos coletados desta espécie foram considerados neríticos, pois eram subadultos ou adultos. Já a tartaruga-de-couro exibe um uso do habitat oceânico na maior parte de sua vida (Bolten 2003), sendo todos os indivíduos coletados considerados oceânicos.

Para avaliar se a ingestão de LPM variou de acordo com o habitat ocupado, também foram comparados os dados brutos de tartarugas-cabeçudas neríticas amostradas entre 2014-2017 (presente estudo) e oceânicas amostradas entre 2007-2009 (Barros 2010). As tartarugas-cabeçudas provenientes de encalhes e capturas incidentais (*bycatch*) das pescarias de arrasto que operam sobre a plataforma continental interna (presente estudo) foram relacionadas à ocupação do habitat nerítico, enquanto que os indivíduos coletados na pescaria de espinhel pelágico na região sul do RS (estudo anterior) foram relacionadas à ocupação do habitat oceânico. Para esta espécie também foi realizada uma análise exploratória das informações pretéritas disponíveis, e as variáveis em comum nestes trabalhos (FO% e volume) foram utilizadas para a comparação da ingestão de LPM. Para avaliar possíveis diferenças na FO% do lixo ingerido entre os habitats foi realizado um teste-t, obedecendo a normalidade dos dados através do teste de Shapiro-Wilk (Royston 1992). Para comparar as médias dos volumes de lixo entre tartarugas neríticas e oceânicas foi realizado um teste de Kruskal-Wallis, devido à falta de normalidade dos dados.

Para avaliar a relação entre a ingestão de LPM e a estratégia alimentar, os indivíduos foram classificados conforme o hábito de alimentação da espécie. Uma vez que algumas espécies de tartarugas marinhas podem apresentar variações na dieta conforme o estágio do ciclo de vida, também classificamos a estratégia alimentar de acordo com esta característica. A tartaruga-verde e a tartaruga-de-pente foram classificadas como onívoras, pois todos os exemplares destas espécies foram de tartarugas no estágio juvenil e nesta etapa da vida alimentam-se tanto de plantas e algas quanto de animais (Bugoni et al. 2003, Grossman et al. 2006, Proietti et al. 2012, Carman et al. 2014). A tartaruga-cabeçuda, tartaruga-oliva e tartaruga-de-couro foram classificadas como carnívoras, pois estas espécies apresentam este tipo de alimentação durante todos os estágios do seu ciclo de vida (Barros 2010, Saba 2013, Colman et al. 2014, Di Beneditto et al. 2015).

2.3.5. Número amostral e ingestão por tamanho do animal

O número de indivíduos analisados neste estudo dependeu das ocorrências de encalhes ao longo da área de estudo (Fig. 1), com algumas das espécies apresentando baixo número de encalhes. Além disso, muitos indivíduos encalhados estavam em avançado estado de decomposição, não sendo possível realizar a amostragem. As análises detalhadas da variedade de LPM e as correlações de tamanho dos indivíduos coletados foram realizadas somente para a tartaruga-verde e tartaruga-cabeçuda, pois foram as espécies que tiveram um maior número de ocorrências (48 e 24 indivíduos amostrados, respectivamente). Gráficos de dispersão par-a-par junto com testes de correlação de Spearman foram feitos para detectar a existência de colinearidade entre as seguintes variáveis: número, massa, volume dos itens ingeridos e a relação entre massa e CCC. O teste mostrou uma alta correlação entre estas quatro variáveis, sendo escolhido para as análises o número de itens por ser a medida mais utilizada entre os trabalhos de ingestão, permitindo assim comparações futuras (Nelms et al. 2015).

Para avaliar se o número amostral representou de forma adequada a variedade de LPM ingerido, foram construídas Curvas de Acumulação de Espécies (Colwell & Coddington 1994). Nesta análise, as ‘espécies’ (eixo y) foram representadas pelos tipos de LPM observados e foram relacionadas ao número de indivíduos amostrados que ingeriram algum tipo de lixo (eixo x). Para avaliar a existência de possível relação entre o tamanho dos indivíduos (CCC) e a quantidade de LPM ingerido foram realizadas análises de correlação de Spearman. Adicionalmente, foram construídos gráficos de dispersão entre o número de itens e o CCC utilizando linhas de regressão pelo método de alisamento *loess* (Cleveland et al. 1991). Dos 48 indivíduos da tartaruga-verde, oito não foram medidos os CCC, portanto foram retirados desta análise.

2.3.6. Importância do tipo e cor de LPM

Para verificar se houve preferência da tartaruga-verde e tartaruga-cabeçuda pela ingestão de determinado tipo e/ou cor de fragmento plástico, foi utilizado um Modelo Linear Generalizado (GLM). A variável resposta foi o número de itens de cada TGI, relacionado com o tipo e a cor de cada fragmento (variáveis explicativas). A distribuição escolhida pertenceu à família Poisson (link = log), pois a utilização desta família permite que a variável resposta seja composta por dados de contagem, com valores iguais ou maiores que zero e a relação da variância média permite heterogeneidade. Foi observada sobredispersão (variância maior do que a média) dos dados, que foi corrigido através do erro padrão utilizando um modelo quasi-GLM (Zuur et al. 2009). O modelo que melhor explicou a variável resposta foi escolhido considerando o melhor ajuste de R^2 (coeficiente de determinação).

Para verificar as diferenças entre tipo e cor dos itens ingeridos por ambas as espécies foram utilizadas duas abordagens: na primeira, calculou-se para cada categoria de tipo e cor de LPM a frequência de ocorrência (FO%), o percentual numérico (N%) e o percentual de massa (M%), para o total de TGIs analisados. A FO% foi calculada pelo número de TGIs contendo a categoria de item ou cor, dividido pelo total de TGIs analisados; o N% foi calculado dividindo o número de itens (abundância) de cada categoria, entre o número total de itens encontrados; e o M% foi calculado dividindo a massa total de itens de cada categoria, entre a massa total de itens encontrados. Na segunda abordagem, calculou-se o Índice de Importância Relativa Presa-específica (%PSIRI, Brown et al. 2012) para determinar a importância de cada tipo e cor na composição do LPM ingerido por cada indivíduo. Este índice leva em consideração a frequência de ocorrência em todos os TGIs (FO%), a percentagem presa-específica numérica (%PN) e a percentagem presa-específica da massa (%PM), pela abundância presa-específica (tipo e cor) de cada indivíduo, conforme a fórmula: %PSIRI = %FO x (%PN + %PM)/2, onde o %PN foi calculado pelo número de vezes que o item (tipo e cor) ocorreu em um TGI dividido pelo número de itens daquele trato. A partir dos valores resultantes para cada item tipo e cor no total de TGIs daquela espécie foi calculada a média, excluindo-se os dados em que aquele item não ocorreu (zeros), resultando na %PN para cada item. O mesmo cálculo foi realizado para calcular a %PM, mas ao invés do número foi utilizada a massa de cada item.

Para as análises de GLM e %PSIRI, foram criadas categorias de itens conforme as características do material, tipo e/ou flexibilidade: fragmentos flexíveis, fragmentos rígidos, linhas de pesca, isopor/espuma, borracha, corda, carvão e outros. Do mesmo modo, foram criadas categorias de cores: amarelo, azul/verde, branco, cinza, colorido, preto/marrom, transparente e vermelho/rosa/laranja. A categorização das cores foi baseada no espectro de comprimentos de onda de luz que as tartarugas marinhas enxergam na água do mar (450 – 620 nm, Bartol & Musick 2003, Fritsches & Warrant 2013).

3. SÍNTESE DOS RESULTADOS

1 – A ingestão de LPM ocorreu nas cinco espécies de tartarugas marinhas, com frequência de ocorrência em 57% dos 86 indivíduos analisados, demonstrando a ampla abrangência da ameaça do LPM a estes animais. A tartaruga-verde apresentou maior FO de ingestão entre as espécies (81,3%), assim como maior variação de itens ingeridos. A tartaruga-cabeçuda apresentou baixa frequência de ingestão de LPM (29,2%). Dois indivíduos juvenis de tartaruga-de-pente foram coletados, sendo que um apresentou ingestão de LPM (50%). Dentre os quatro indivíduos de tartaruga-de-couro, um deles ingeriu LPM (25%). A tartaruga-oliva apresentou menor ingestão de LPM dentre as espécies analisadas (12,5%).

2 – Praticamente um terço dos TGIs da tartaruga-verde (31,3%) encontravam-se obstruídos por algum fragmento e/ou havia formação de fecalomas no intestino. Na tartaruga-cabeçuda, um indivíduo apresentou obstrução no TGI.

3 – Cinco categorias de composição de itens foram identificadas (plástico, borracha, madeira, papel e tecido), sendo que os plásticos foram os mais ingeridos por todas as espécies, apresentando elevada frequência, número de fragmentos e massa. Dentre os itens plásticos, as embalagens, linhas de pesca, fragmentos flexíveis e rígidos foram os mais ingeridos pelas espécies; e os balões foram os mais ingeridos pela tartaruga-verde dentre os itens não plásticos.

4 – A composição polimérica dos itens mais ingeridos pelas tartarugas marinhas foi identificada, em ordem decrescente de ocorrência, como: polietileno (PE), poliamida (PA), poli acetato-vinilo de etileno (EVA), poliuretano (PUR), polipropileno (PP), poli acronitrila butadieno estireno (ABS) e poliestireno (PS).

5 – A comparação temporal da ingestão de LPM pela tartaruga-verde apresentou FO da ingestão com valores iguais ou acima de 70% na maior parte dos anos analisados e em alguns anos ocorreu em 100% dos indivíduos. A massa dos resíduos ingeridos em média foram semelhantes ao longo dos anos, com exceção de 2010 que foi elevada.

6 – Com relação ao habitat, a ingestão de LPM foi alta tanto para os indivíduos considerados neríticos quanto para os oceânicos. Na comparação entre habitats pelas tartarugas-cabeçudas, os indivíduos oceânicos apresentaram maiores valores de ingestão de LPM do que os indivíduos neríticos, tanto em FO% quanto em volume. Com relação à estratégia alimentar, os indivíduos considerados onívoros apresentaram maior ingestão do que os carnívoros.

7 – Para as tartarugas-verdes amostradas, a quantidade total de itens ingeridos não apresentou correlação significativa com o tamanho (CCC). Para a tartaruga-cabeçuda foi observada uma correlação significativa negativa entre o número de itens ingeridos e o tamanho da tartaruga, sendo observado que indivíduos com CCC $\geq$ 70 cm não ingeriram lixo.

8 – Para a tartaruga-verde houve interação entre tipo e a cor dos itens ingeridos e, portanto, estas características foram analisadas em conjunto, resultando em 39 combinações. As categorias dos itens com maiores valores de FO%, %N, %M foram os fragmentos flexíveis transparentes, flexíveis brancos e rígidos brancos. Estes também foram os que tiveram maior importância na ingestão desta espécie, com PSIRI = 28,9%, 12,4% e 11,5%, respectivamente. Os fragmentos flexíveis transparentes também tiveram os maiores valores de FO% (89,74), %N (25,1) e %M (14,8). Dentre as demais categorias, os fragmentos rígidos brancos tiveram maiores valores de número e massa que os fragmentos flexíveis brancos, porém foram menos frequentes. Por isso a importância de ambos na ingestão foi semelhante para os fragmentos rígidos brancos (%PSIRI = 11,5) e para os flexíveis brancos (%PSIRI = 12,4). Outros itens ingeridos

por esta espécie com frequência e em maior quantidade foram os fragmentos flexíveis preto/marrom, linhas azul/verde, isopor branco, fragmentos rígidos transparentes, rígidos preto/marrom e rígidos azul/verde.

9 – Para a tartaruga-cabeçuda, não houve interação entre tipo e cor dos itens, sendo estes analisados em separado. Os tipos mais ingeridos em quantidade por esta espécie foram os fragmentos rígidos: FO% = 42,9, %N = 23,5, %M = 47,3. Os fragmentos flexíveis e os itens de isopor/espuma apesar de alta frequência (FO% = 42,9 e 28,6, respectivamente) tiveram menor representatividade em %N e %M. As linhas foram muito mais numerosas (41,2%) e a borracha teve mais massa (40,8%) no total de itens ingeridos pela espécie. A importância de cada tipo por indivíduo foi principalmente de fragmentos rígidos (%PSIRI = 29,6) na ingestão por esta espécie, seguido do isopor (18,3) e dos fragmentos flexíveis (17,7). As cores mais ingeridas por esta espécie foram branca (FO% = 57,1, %N = 26,6, %M = 57,8) e os itens de cor preto/marrom (FO% = 42,9, %N = 17,6, %M = 2,9), apresentando também uma maior importância na ingestão (%PSIRI = 40,1 e 29,4, respectivamente). As cores da categoria azul/verde apresentaram elevados valores em número (32,4%) e a categoria vermelho/rosa/laranja, em massa (35,7%).

4. CONCLUSÕES

A ingestão de LPM por todas as espécies de tartarugas marinhas que ocorrem na costa brasileira representa a grande ameaça do LPM ao meio marinho, já que a poluição por estes materiais vem aumentando sua magnitude e consequente aumento das interações não naturais com a biota. A tartaruga-verde apresentou maior abundância e diversidade de itens plásticos ingeridos dentre as espécies e esta ingestão tem permanecido elevada e constante pelo menos ao longo dos últimos 20 anos na região e, portanto, a ingestão de LPM por juvenis desta espécie é um grave problema. Fragmentos de itens descartáveis e de pesca foram amplamente ingeridos pelas espécies, o que pode indicar maior disponibilidade destes na água do mar.

As características biológicas e ecológicas das espécies de tartarugas marinhas determinam o grau de interação com o LPM assim como a vulnerabilidade ao LPM presente no ambiente marinho. As tartarugas de alimentação onívora estão mais suscetíveis à ingestão do LPM devido a sua alimentação generalista e oportunista em comparação às carnívoras. Apesar de o habitat oceânico ser considerado o local de maior probabilidade de ingestão de LPM por tartarugas marinhas, no habitat nerítico pode ter elevadas quantidades de LPM e assim propiciar a ingestão ao mesmo nível. A ingestão de LPM pelas tartarugas-cabeçudas foi maior no ambiente oceânico do que no nerítico, possivelmente devido a diferenças na estratégia de alimentação entre os locais. As tartarugas-verdes parecem ingerir LPM de forma constante quando juvenis entre 30-60 cm, ou retém mais facilmente os itens ingeridos nos seus TGIs, enquanto que as tartarugas-cabeçudas ingerem LPM até 70 cm e quando maiores que este tamanho tem menor ingestão ou maior capacidade de eliminação do LPM ingerido.

A ingestão de fragmentos flexíveis e de cores claras por juvenis da tartaruga-verde, possivelmente ocorreram por serem mais atraídas por estes itens ou maior disponibilidade onde se alimenta. Entretanto, foram ingeridos fragmentos rígidos em quantidades elevadas, assim como itens de cores variadas, representando uma alimentação oportunista e generalista neste estágio do ciclo de vida. A ingestão de fragmentos rígidos e de cores claras e escuras pela tartaruga-cabeçuda pode estar associada à semelhança às características dos itens alimentares ingeridos com o LPM, assim como maior especialização alimentar da espécie na região nerítica.

O conhecimento e entendimento da ingestão de LPM pelas diferentes espécies de tartarugas marinhas é uma grande prioridade, pois é importante para fornecer bases para a definição de medidas públicas de mitigação da poluição marinha. Entre algumas medidas que podem ser aplicadas para a redução deste problema estão: identificação das fontes principais de resíduos sólidos e formas de redução do descarte destes materiais; restrição ou redução de plásticos descartáveis, principalmente próximos ou em praias, rios e lagos, ambientes ao ar livre como parques ou campos; maior valor sobre itens descartáveis de uso único tanto para as empresas quanto para os consumidores, assim como utilização de garrafas e embalagens retornáveis, gerando economia no produto sem a embalagem; e não menos importante, a implementação de campanhas governamentais de incentivo para a redução, reutilização e reciclagem do lixo em geral.

5. REFERÊNCIAS BIBLIOGRÁFICAS

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6. APÊNDICE

Ingestion of plastic marine litter by sea turtles in southern Brazil: abundance, characteristics and selectivity

Manuscrito redigido de acordo com as normas de submissão ao periódico
Marine Pollution Bulletin – Capes QUALIS A1

Ingestion of plastic marine litter by sea turtles in southern Brazil: abundance, characteristics and selectivity

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Abstract

The ingestion of plastic marine debris (PML) by sea turtles is widespread and concerning. All sea turtle species are threatened and continuously vulnerable to this type of pollution. In this work, we quantified and characterized PML ingested by five sea turtle species that occur in Brazil – green *Chelonia mydas*, loggerhead *Caretta caretta*, olive ridley *Lepidochelys olivacea*, leatherback *Dermochelys coriacea* and hawksbill *Eretmochelys imbricata* – according to the biology and ecology of species and characteristics of PML, and evaluated the ingestion of such litter by green turtles over time. Gastrointestinal tracts were collected between 2013 and 2017 from turtles stranded dead on the beach and captured as bycatch in fisheries. Ingestion of PML was observed in 49 out of the 86 individuals (57.0%) and occurred in all species; the green turtle showed the highest intake (81.3%). The intake of PML by green turtles has been high and constant since 1997 in the area. Fragments of disposable items and fishing gear were the most abundant items in the sea turtle tracts. Omnivorous individuals presented higher PML intake than carnivorous ones. Neritic and oceanic sea turtles considered neritic and oceanic showed similar PML ingestion rates. A significant negative correlation between the number of ingested items and size was observed for loggerhead, but not for green turtles. Flexible transparent fragments, flexible white and hard white were the main items ingested by the green turtles; hard, flexible and closed-cell extruded polystyrene foam/foam were the main types, while white and black/brown were the main colors ingested by loggerheads. These results help us better understand PML ingestion by different sea turtle species, showing that it is a continuous and elevated threat for these endangered animals, and provide baseline information for the definition of prevention and mitigation strategies for marine plastic pollution.

Keywords: marine plastic pollution, marine turtles, Western South Atlantic, impacts, temporal analysis, selectivity index.

1. Introduction

Pollution caused by plastic marine litter (PML) is currently one of the most widespread and long-lasting impacts to the oceans and marine life (Derraik, 2002; Barnes et al., 2009; Gregory, 2009). PML are solid debris of anthropogenic origin that enter marine and coastal environments (Coe and Rogers, 2000), and represent the most common type of pollutant in interior and oceanic waters worldwide (Sheavly and Register, 2007). This type of debris accumulates from the poles to the equator, from the sea surface to the seafloor, and even at remote areas such as Antarctica and oceanic islands located thousands of kilometers from the coast (UNEP, 2009; Barnes et al., 2009; Cózar et al., 2014). PML can be land-based (main source) from urban, industrial and touristic activities and be carried by sewage, fluvial systems, surface runoff and winds to beaches and oceans; or sea-based, resulting from fishing activities, oil extraction facilities, and tourist, commercial, military and research vessels (Ivar do Sul and Costa, 2007; Sheavly and Register, 2007). Because PML are long-lasting, i.e. had slow degradation rates, they accumulate at sea. Accumulation regions of PML are varied and influenced by factors such as proximity to urban areas, maritime activities and predominant oceanographic conditions (e.g. gyres, currents, fronts; Galgani et al., 2015).

Historically, impacts caused by PML have been directly related to the development of synthetic polymers, popularly known as plastics (UNEP, 2016), which constitute approximately 90% of litter present in coastlines and the oceans (Derraik, 2002; Thompson et al., 2009; Ryan, 2014). Plastic production has grown exponentially since the 1930s (Thompson et al., 2009), and between the years 2000 and 2016 increased from 200 to 335 million tons per year (Plastics Europe, 2017). Based on Jambeck et al. (2015), it is estimated that between 5.7 and 15.4 million tons of plastics entered the oceans in 2016, mainly due to inadequate destination and waste management. The increase in production along with inadequate discard is leading to increased concentrations of plastics at sea, and consequently resulting in higher availability and probability of interactions with the marine animals. These interactions can threaten marine biota in several ways, with ingestion being one of the most commonly reported impacts: at least 395 species of several animal groups have been reported to suffer impacts due to the ingestion of plastics (Gall and Thompson, 2015).

PML ingestion can result in sublethal or lethal effects in animals, including sea turtles (Oehlmann et al., 2009). Sublethal effects include internal lesions, gastrointestinal tract blockage, weakness, emaciation and buoyancy alterations (Gregory, 2009; Oehlmann et al., 2009). Additionally, PML ingestion can lead to impacts along food webs, since additives and pollutants associated with plastics can bioaccumulate and biomagnify upward to top predators (Reisser et al., 2014). The first record of PML ingestion by sea turtles was reported in the 1980s (Balazs, 1984; Carr, 1987), and since then, has been increasingly reported (Schuyler et al., 2014a; Nelms et al., 2015). In a global analysis, it was noted that 96.8% of thirty studies reported litter ingestion (Schuyler et al., 2014a). This ingestion has been recorded for all seven extant sea turtle species (Kühn et al., 2015), representing a serious threat to these threatened animals. All sea turtle species are listed by the International Union for the Conservation of Nature’s Red List (IUCN, 2018), and the five that occur along the Brazilian coast are currently classified as: vulnerable (loggerhead - *Caretta caretta*, leatherback - *Dermochelys coriacea* and olive ridley - *Lepidochelys olivacea*), endangered (green - *Chelonia mydas*) and critically endangered (hawksbill - *Eretmochelys imbricata*). In Brazil, populations are classified according to the Brazilian List of Threatened Species

(MMA, 2014) as vulnerable (green), endangered (loggerhead and olive ridley) and critically endangered (leatherback and hawksbill).

Litter ingestion can occur in different ways in the marine environment: directly, when the animal actively selects the material due to similarity with their prey (e.g. plastic bags and balloon mistaken for gelatinous animals) (Schuyler et al., 2012; Hoarau et al., 2014; Schuyler et al., 2014b); and indirectly, when litter is accidently ingested during prey consumption (e.g. green turtles grazing algae and seagrass beds) or in ingestion of barnacles and other adhered organisms (Di Beneditto and Awabdi, 2014). Therefore, the ingestion of litter by sea turtles can be influenced by the habitat they occupy as well as their diet preferences throughout their life stages, according to availability of PML at different environments and the foraging strategies of animals (Schuyler et al., 2014a). A generalized sea turtle life cycle includes an oceanic phase after hatchlings are born and enter the sea, when small turtles can remain in the surface oceanic gyres foraging opportunistically and being susceptible to the ingestion of floating plastic particles (Schuyler et al., 2012). After reaching recruitment size, some species remain in the oceanic environment while others recruit to neritic zones (Bolten, 2003), where they generally adopt a benthic feeding strategy and can encounter PML due to the proximity to urban centers (Schuyler et al., 2012). In terms of feeding strategy, sea turtles present interspecific differences, as well as intraspecific variations according to life cycle stage: post-hatchlings and early juveniles tend to be more generalist, becoming more specialized in their diets when large juveniles and adults, which could result in lower probabilities of PML ingestion (Schuyler et al., 2014a).

The diet of juvenile green turtles is composed mainly by seaweed, seagrasses, mollusks, fish, anemones and gelatinous animals (Bjorndal et al., 1997; Bugoni et al., 2003; Carman et al., 2014). Hawksbill sea turtles feed mainly on sessile benthic animals such as zoanthids and sponges, but can also ingest seaweed, coelenterates and crustaceans (Grossman et al., 2006; Proietti et al., 2012; Bell, 2013). Loggerhead turtles, when in the oceanic environment, feed on gelatinous organisms such as salps and pyrosomes, and in the neritic environment on benthic invertebrates such as crustaceans and mollusks, and fish (Barros, 2010; Di Beneditto et al., 2015). Olive ridleys feed mainly on fish, crustaceans and mollusks (Colman et al., 2014; Di Beneditto et al., 2015), and can also ingest gelatinous organisms in oceanic areas (Di Beneditto et al., 2015). The diet of leatherback turtles is composed of gelatinous animals such as jellyfish, pyrosomes and ctenophores (Saba, 2013).

All sea turtle species that occur in Brazil have been reported stranded on the coast of Rio Grande do Sul (RS) state, which adjacent waters represent an important feeding ground for green, loggerhead and leatherback turtles (Monteiro et al., 2016). Sea turtles become stranded along this coast throughout the entire year, but with higher frequencies in the austral spring and summer (October to March; Monteiro et al., 2016). In this region an increase in the number of stranded loggerheads and greens was observed (from dozens to hundreds of animals per year), as well as an increase in records of olive ridleys and hawksbills (Monteiro et al., 2016). PML ingestion has been analysed for some sea turtle species in the area: over 60% of stranded green turtles (Bugoni et al., 2001; Tourinho et al., 2010) and over 90% of loggerheads captured as bycatch in oceanic fisheries have been reported to ingest PML; however, stranded loggerheads showed low PML ingestion (Barros, 2010). Leatherbacks have also been shown to ingest PML, with two occurrences registered for five individuals (Pinedo et al., 1996; Bugoni et al., 2001).

Understanding the degree of vulnerability of species to litter present in the oceans is crucial to define effective prevention and mitigation strategies for these flagship species (Eckert and Hemphill, 2005; Nelms et al., 2015). Although the impacts of PML ingestion by sea turtles have been well described, the factors that lead to this type of interaction are still unclear. Investigating how different sea turtle species ingest PML allows the identification of common patterns of litter ingestion of species that possess distinct diets and habitat uses. PML ingestion by sea turtles is not easy to evaluate when considering their continuous distribution and complex life cycles; however, this evaluation is among the research priorities for these animals (Vegter et al., 2014).

In this context, this work evaluated PML ingestion by the five sea turtles that occur in the southern of Brazil. We hypothesize that due to the increasing amounts of PML in the oceans, all sea turtle species will present litter ingestion, and that these rates have increased over time. Objectives associated with these hypotheses were to: i) verify and quantify PML occurrence in the gastrointestinal tracts of sea turtles along the coast of RS and ii) compare PML ingestion by green turtles over time (1997-2017). We also hypothesize that sea turtles with feeding more specialist, that use the oceanic habitat and the smaller sizes present a highest quantity and frequency of PML ingested and the PML items most similar the sea turtles preys are more ingested. Goals associated with this hypothesis were to: i) characterize ingested PML; ii) verify the influence of species, habitat, diet and turtle size on this ingestion; and iii) evaluate if sea turtles ingested PML according to its characteristics.

2. Material and methods

2.1. Study area

Gastrointestinal tracts (GIT) evaluated in this study were sampled from sea turtles stranded along the southern coast of Rio Grande do Sul state (RS), covering approximately 350 km of beach from Lagoa do Peixe (31°20'S; 51°05'W) to Arroio Chuí (33°45'S; 53°22'W). Additionally, GITs were obtained from turtles incidentally caught in pair trawl fisheries over the internal continental shelf close to the Lagoa dos Patos estuary mouth (Fig. 1). The RS coast presents a northeast-southwest orientation, with continuous sandy beaches dominated by waves (Calliari et al., 2005). The oceanic region is influenced by the southern-flowing Brazil Current at the western border of the Subtropical Gyre, and the Falklands Current, that flows to the north along the western border of the Argentinean Basin. These currents meet at approximately 38°S, creating the Brazil-Falklands Convergence Zone (Oliveira et al., 2009). Part of the waters formed by this interaction flows to the north over the Uruguayan and south Brazilian shelf, mixing with waters from the Río de La Plata (34°58'S; 54°57'W) and Lagoa dos Patos and forming the Subtropical Shelf Front. This front is a complex thermohaline structure that extends towards the north during Austral winter and to the south during summer (Piola et al., 2000). The dynamic interaction of these different water masses creates an area with elevated nutrients and primary/secondary production that sustains important fishing activities (Muelbert et al., 2008) and leads to the concentration of numerous marine species, including sea turtles.

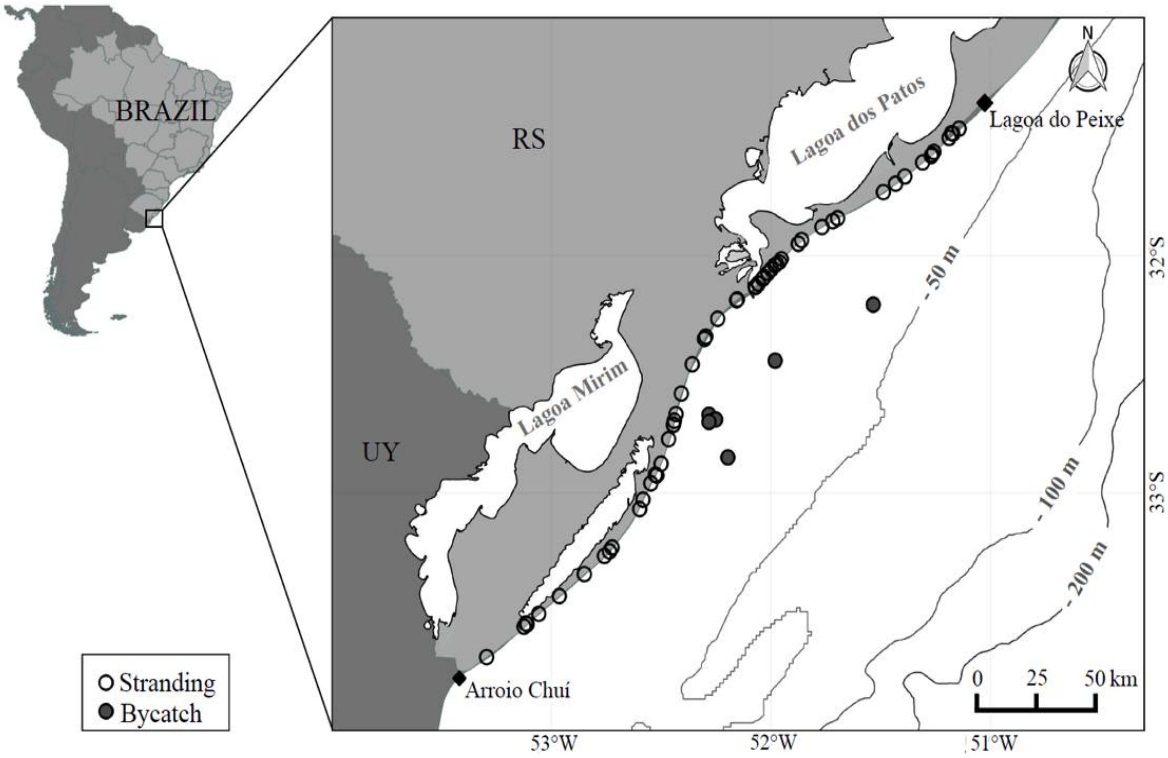

Fig. 1. Study area with sampling locations (circles) along the southern Rio Grande do Sul coast. RS = Rio Grande do Sul, Brazil; UY = Uruguay.

2.2. Sampling and sample processing

We obtained 86 sea turtle GITs, with 80 originating from beach monitoring conducted from May 2013 to November 2017 by the *Núcleo de Educação e Monitoramento Ambiental* (NEMA – Environmental Education and Monitoring Nucleus). NEMA on-board observers sampled six additional individuals that were incidentally caught in pair trawls over the internal continental shelf off the study area. For each turtle, we recorded curved carapace length (CCL) in cm, measured from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supracaudals; CCL for leatherbacks was measured from the nuchal notch (anterior edge of the carapace at the midline) to the posterior tip of the caudal peduncle, alongside the vertebral ridge (Bolten, 1999). Date and information on the stranding and capture point (latitude and longitude) of each animal were also recorded. Turtles found dead were necropsied in the field, with each GIT frozen for posterior analysis. Live animals were taken to the *Centro de Recuperação de Animais Marinhos* (CRAM – Marine Animal

Recovery Centre from Universidade Federal do Rio Grande – FURG) for rehabilitation, and necropsied after death.

GITs were removed by incision from the cranial portion of the esophagus to the final portion of the large intestine. The GITs (esophagus, stomach and intestine) was evaluated and the organic material (diet items) was separated from inorganic items, which were washed in a sieve with 1 mm mesh. To quantify the proportion of PML in relation to organic material, both were weighed (wet mass) in a precision scale (0.1 g precision). The presence of obstructions to the GITs was recorded for posterior evaluation of possible impacts caused by PML. We considered a GIT obstruction when a fragment was stuck to the GIT wall or had fecalomas (hard fecal material). After washing the material, PML items were dried in a dry oven at 60°C for 2 to 10 h, and each item was quantified, mass measured (g), measured for volume (length × width × height - cm^3) and characterized according to the United Nations Environmental Program in terms of material, type, color and flexibility (UNEP, 2009).

To determine the polymer type of ingested plastics, in order to verify the main origin, we randomly selected 128 fragments of ingested PML and cut a small piece of 3 × 3 mm. Sampled were dried in a dry oven at 40°C for three to seven months, depending on the humidity of the sample. Polymer composition was identified through Fourier Transform Infrared Vibrational Spectroscopy (FTIR), according to the ATSM SP E1252 – 98 normative using a Prestige 21 spectrometer, with a diffuse reflectance module, 24 sweeps and 4 cm^{-1} resolution. Peak analysis was done considering only signs with over 50% intensity and considering the most intense peak. The spectra generated from samples were compared to those of known plastic polymers (Silverstein et al., 2007) to identify the polymers and their degradation signs. These analyses were performed at “Escola de Química e Alimentos (EQA-FURG)”.

2.3. Data analyses

2.3.1. PML ingestion parameters

PML ingestion by sea turtles was evaluated through the following parameters of items found by species, individuals and part of the GIT: number of items (N), mass in grams (M) and volume in cm^3 (V). The relative Frequency of Occurrence (FO%), referring to the percentage of occurrence of PML in relation to the total number of analysed contents, was calculated for each species and portion of the GIT.

2.3.2. Sea turtle life cycle stages

Sampled individuals were classified according to the life cycle stage, in juveniles or adults (Table 1). This classification was based on the minimum CCLs of females at the largest nesting areas in Brazil, as suggested by Monteiro et al. (2016). Turtles smaller than these minimum sizes were considered juveniles, and those larger were considered adults.

Table 1. Curved carapace length (CCL) and life stage of sampled sea turtles at southern Brazil between 2013 and 2017. SD = standard deviation.

Species	Sample origin	CCL min - max (cm) (mean ± SD)	Minimum nesting CCL (cm)	Life stage
Chelonia mydas (n = 48)	Stranded	28 - 60 (39 ± 7)	90[a]	juveniles
Caretta caretta (n = 24)	Stranded Bycatch	40 - 107 (73.5 ± 15)	83[b]	19 juveniles, 5 adults
Lepidochelys olivacea (n = 8)	Stranded Bycatch	62 - 72 (67.9 ± 3.5)	63[c]	1 juveniles, 7 adults
Dermochelys coriacea (n = 4)	Stranded	107 - 170 (141 ± 22)	139[d]	2 juveniles, 2 adults
Eretmochelys imbricata (n = 2)	Stranded	33 – 37	86[e]	juveniles

[a]Almeida et al., 2011a; [b]Baptistotte et al., 2003; [c]Silva et al., 2007; [d]Thomé et al., 2007; [e]Marcovaldi et al., 1999.

2.3.3 Temporal comparison of PML ingestion by green turtles

The temporal variability of PML ingestion by green turtles was evaluated by comparing our data with those of previous studies in the region. Data on PML intake by green turtles were provided by the study authors, and comprised the following periods: 1997 (Bugoni et al., 2001), 2006-2007 (Tourinho et al., 2010), 2010 (Ruzzene, 2001), 2011-2014 (Colferai et al., 2017) and 2013-2017 (present study). No data was available for 1999 to 2005 and 2008-2009. We highlight that in 1997 only the esophagus and stomach were evaluated, and therefore the ingestion of plastics is likely underestimated.

Initially, we performed an exploratory analysis of the previous data, which was reorganized and standardized in spreadsheet according to the information of each study. In this way, it was possible to identify the variables in common for the five periods: year of collection, presence/absence of PML in GITs and mass (g) of litter, per individual. To analyse variation of PML ingestion over time, the FO% of ingestion and the mass of ingested litter per individual were calculated for each year. In 1997 (Bugoni et al. 2001), only the PML found in esophagus and stomach was evaluated. Therefore, it was made a correction to all the GIT, based on Colferai et al. (2017) and present study data, for: mass, calculated by the percentage that the esophagus and stomach represented the total PML and extrapolating to the whole GIT; and FO%, by increasing of FO%, when was calculated to the esophagus and stomach in relation to total GIT, considering the GIT in which there was only PML in intestine. Data did not comply with the normality assumption verified through the Shapiro-Wilk test (Royston, 1992) and the comparison of PML mass between years was performed through a Kruskal-Wallis test. Then, a non-parametric Dunn's test (Dunn 1964) was performed for pairwise comparisons between the average masses.

2.3.4. Habitat and feeding strategy

In order to evaluate the relation between PML intake and occupied habitat, individuals were classified into two groups: neritic and oceanic. For the green and loggerhead turtle, the habitat was classified based on the size of the first recruitment for the neritic environment, which for the region is 30 cm for the green turtle (Lenz et al., 2016) and 55 cm for the loggerhead turtle (Lenz et al., 2017). Hawksbill turtle habitat was classified based on the size of the first recruitment for the Atlantic (Meylan, 1988) and Puerto Rico (Diez & van Dam, 2002), with 20 cm being the minimum size for both locations. West Atlantic Ocean turtle populations recruit to the neritic region after part of their development in the ocean environment (Reichart, 1993; Bolten, 2003; Silva et al., 2011). Thus, the individuals collected of this species were considered neritic since they were subadults or adults. The leatherback turtle, however, exhibits a use of oceanic habitats for most of its life (Bolten, 2003), with all collected individuals being considered oceanic.

Additionally, we compared PML ingestion data from neritic loggerheads sampled between 2014 and 2017 (present study) and oceanic individuals sampled between 2007 and 2009 (Barros, 2010). Loggerhead turtles from beach strandings and incidentally captured by pair trawl fleets were related to the occupation of the neritic habitat, while individuals collected in the pelagic longline fishery in the southern region of RS (previous study) were related to the oceanic habitat. An exploratory analysis of the available information was also performed, and the common variables in these studies (FO% and volume) were used to compare PML intake. In order to evaluate possible differences in FO% of PML ingested between habitats, a Student's t-test was performed, after checking for data normality through the Shapiro-Wilk test (Royston, 1992). A Kruskal-Wallis test was performed to compare the mean litter volumes between neritic and oceanic turtles, due to the lack of normality of the data.

To evaluate the relation between PML intake and diet strategy, individuals were classified according to the feeding habits of the species. Since some species of sea turtles can present variations in diet according to the life cycle stage, we also classified the strategy according to this characteristic. Green and hawksbill turtles were classified as omnivorous, as all specimens were juveniles, and at this stage they feed on plants, algae and animals (Bugoni et al., 2003; Grossman et al., 2006; Proietti et al., 2012, Carman et al., 2014). Loggerheads, olive ridleys and leatherbacks were classified as carnivorous, as these species present this type of feeding during all stages of their life cycle (Barros, 2010; Saba, 2013; Colman et al., 2014; Di Beneditto et al., 2015).

2.3.5. Sample size and ingestion by animal size

The number of individuals analysed in this study depended on the occurrence of strandings along the study area (Fig. 1), with some species presenting low number of stranded individuals. Additionally, many stranded individuals were in an advanced state of decomposition, and sampling was not possible. Therefore, a detailed analysis of ingested PML variety and the size correlations of sampled individuals were performed only for the green and loggerhead turtle, as these species had the highest number of occurrences (48 and 24 individuals, respectively). Paired dispersion plots along with Spearman's correlation tests were done to detect co-linearity between the following variables: number, mass, volume of ingested items and the relationship between mass and CCL (Supplementary material Figure S1). The test showed a high correlation between these four variables, and the number of ingested items was chosen for analyses since this is the most used measure among ingestion studies, thus allowing future comparisons (Nelms et al., 2015).

Species Accumulation Curves were constructed (Colwell and Coddington, 1994) were constructed to evaluate if the sample number adequately represented the variety of ingested litter. In this analysis, the species were represented by the types of observed PML (y-axis) and were related to the number of sampled individuals that ingested some type of litter (x-axis). Spearman correlation analyses were performed to evaluate the relationship between the size of the individuals (CCL) and the amount of ingested PML. Additionally, scatter plots between the number of items and the CCL were done using regression lines by the loess smoothing method (Cleveland et al., 1991). Of the 48 sampled green turtles, eight did not have their CCL measured, thus were removed from analysis.

2.3.6. Importance of type and color of PML

In order to verify if there was a preference of green and loggerhead turtles for ingesting a certain type and/or color of plastic fragments, a Generalized Linear Model (GLM) was used. The response variable was the number of items of each GIT, related to the type and color of each fragment (explanatory variables). The chosen distribution belonged to the Poisson family (link = log), since the use of this family allows the response variable to be composed by count data, with values equal to or greater than zero and the mean variance ratio allowing heterogeneity. Overdispersion of the data was observed, which was corrected through the standard error using a quasi-GLM model (Zuur et al., 2009). The model that best explained the response variable was chosen considering the best R^2 adjustment.

To verify the differences between type and color of items ingested by both species, two approaches were used: first, the relative frequency of occurrence (FO%), numerical percentage (%N) and mass percentage (%M), for the total GITs analysed. The FO% was calculated by the number of GITs containing the category of item or color, divided by the total number of analysed GITs; the %N was calculated by dividing the number of items (abundance) of each category, by the total number of items found; and %M was calculated by dividing the total mass of items in each category by the total mass of items found. In the second approach, the Prey-Specific Index of Relative Importance (%PSIRI; Brown et al., 2012) was calculated to determine the importance of each type and color of items of PML ingested by each turtle. This index takes into account the frequency of occurrence in the GIT (FO%), the prey-specific numerical percentage (%PN) and the prey-specific mass percentage (%PM). The prey-specific abundance (type and color) was then calculated according to the formula: %PSIRI = %FO x (%PN + %PM)/2, where %PN was calculated by the number of items of a certain type and color that occurred in a GIT, divided by the number of items of the respective GIT. Based on the resulting values of each type and color in the total number of TGIs for a determined species, we calculated the mean (excluding zeros), which led to the %PN for each item. The same calculation was done to obtain the %PM, using the mass of each type and color of items.

For the GLM and PSIRI analyses, item categories were created according to the characteristics of the material, type and/or flexibility: flexible fragments, rigid fragments, fishing lines, closed-cell extruded polystyrene foam (XPS)/foam, rubber, rope, coal and others. In the same way, we created color categories: yellow, blue/green, white, gray, colorful, black/brown, transparent and red/pink/orange. The color categorization was based on the spectrum of light wavelengths that sea turtles see in seawater (450 - 620 nm; Bartol and Musick, 2003; Fritsches and Warrant, 2013).

3. Results

3.1. Characteristics of PML ingestion

Litter ingestion was recorded in 49 out of the 86 sampled sea turtles (57.0%) and for all five species, with the green turtle showing the highest frequency of occurrence (FO% = 81.3) and the olive ridley presenting the lowest (FO% = 12.5; Table 2). The number of items per individual ranged from 1 to 544 for the green and from 1 to 19 for the loggerhead turtle. In the hawksbill turtles two items were found in one individual, and in olive and leatherback turtles one item was found in one individual per species.

Table 2. Summary of the frequency of occurrence (FO%) and number of fragments (N) of ingested plastic marine litter of sampled sea turtles at southern Brazil between 2013 and 2017. SD = standard deviation.

Species	FO%	N min-max (mean ± SD)
Chelonia mydas (n = 48)	81.3	1 - 544 (57.4 ± 89.8)
Caretta caretta (n = 24)	29.2	1 - 19 (1.4 ± 4.0)
Lepidochelys olivacea (n = 8)	12.5	1
Dermochelys coriacea (n = 4)	25.0	1
Eretmochelys imbricata (n = 2)	50.0	2

Among the analysed sea turtle species, only the green and loggerhead turtle presented obstructions of the GIT caused by the ingestion of PML. In the green turtle, 31.3% of the GITs were obstructed by fragments and/or had fecalomas formed in the intestine. When there was an obstruction, the mass of the observed items varied from 8 to 574.3 g (wet mass) and in some of these cases, lesions on the wall of the intestine could be observed (see photos in Supplementary Material - Figure S2). In these individuals, the wet mass of litter contributed from 1.0 to 87.0% of the wet mass of the total stomach contents. In the loggerhead turtle, a fecaloma was observed in the intestine of one individual, containing 11.1 g (wet mass) of plastics in this portion of the GIT.

Five PML categories in terms of constituent material were identified in the GITs of the five sea turtle species (plastic, rubber, wood, paper, cloth). Plastics were ingested by all species, presenting the highest occurrence, abundance and mass (Table 3). Non-plastic materials (rubber, wood, paper and cloth) were found only in green and hawksbill turtles. The green turtle presented the largest variety of ingested plastic items, with the highest frequency of occurrence corresponding to packaging (FO% = 82.1), fishing lines (FO% = 76.9) and hard fragments (FO % = 74.4), with the latter presenting the highest number (N = 1108) and mass (M = 208.8 g). Non-plastic items ingested by this species, party balloons had the highest frequency of occurrence (FO% = 48.7). For the loggerhead turtle, hard fragments presented the highest frequency of occurrence (FO% = 42.9) and mass (M= 3.4 g) and fishing lines highest number (N = 14) (Table 3).

Table 3. Frequency of occurrence (FO%), number of fragments (N), mass (M) and volume (V) of different types of plastic marine litter ingested by sea turtles in South Brazil, between 2013 and 2017.

		Chelonia mydas Green (n = 39)				*Caretta caretta* Loggerhead (n = 7)				*Eretmochelys imbricata* Hawksbill (n =1)				*Lepidochelys olivacea* Olive ridley (n =1)				*Dermochelys coriacea* Leatherback (n = 1)			
Material	**Type**	**FO%**	**N**	**M (g)**	**V (cm^3)**	**FO%**	**N**	**M (g)**	**V (cm^3)**	**FO%**	**N**	**M (g)**	**V (cm^3)**	**FO%**	**N**	**M (g)**	**V (cm^3)**	**FO%**	**N**	**M (g)**	**V (cm^3)**
Plastic	Packaging	82.0	552	36.2	173.4	--	--	--	--	--	--	--	--	--	--	--	--	100	1	<0.1	0.7
	Fishing lines	76.9	171	4.0	30.9	14.3	14	0.1	<0.1	--	--	--	--	100	1	<0.1	<0.1	--	--	--	--
	Hard fragments	74.4	1108	208.8	504.2	42.9	8	3.4	5.9	--	--	--	--	--	--	--	--	--	--	--	--
	Bags	66.7	173	15.7	65.1	14.3	1	<0.1	0.7	--	--	--	--	--	--	--	--	--	--	--	--
	Soft fragments	53.8	381	31.2	316.8	28.6	2	<0.1	0.4	100	2	<0.1	0.1	--	--	--	--	--	--	--	--
	Closed-cell extruded polystyrene foam	41.0	33	1.8	20.3	14.3	2	<0.1	0.3	--	--	--	--	--	--	--	--	--	--	--	--
	Entangled wires	38.5	59	15	269.1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Synthetic fibers	38.5	53	12.4	117.6	14.3	3	0.5	4.6	--	--	--	--	--	--	--	--	--	--	--	--
	Straws	33.3	16	1.3	4.6	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Ropes	33.3	32	6.7	29.2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Cups	25.6	22	2.5	12.2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Foam	17.9	33	6.4	216.7	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Mesh bag fragments	12.8	16	0.7	1.2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Resin pellets	12.8	23	0.4	0.8	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Bottle caps	10.3	7	1.8	5.7	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Cigarette butts	2.6	1	<0.1	0.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Rubber	Baloons	48.7	39	8.1	17.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Rubber fragments	28.2	14	2.9	9.6	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Gloves	2.6	1	0.4	0.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Rubber bands	2.6	1	<0.1	0.1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Footwear fragments	--	--	--	--	14.3	1	2.9	25.9	--	--	--	--	--	--	--	--	--	--	--	--
Wood	Charcoal	23.1	16	2.8	7.7	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Paper	Cardboard	--	--	--	--	14.3	3	0.2	1.5	--	--	--	--	--	--	--	--	--	--	--	--
Cloth	Cloth fragments	5.1	3	0.5	3.9	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
TOTAL			**2754**	**359.6**	**1807.6**		**34**	**7.2**	**39.4**		**2**	**<0.1**	**0.1**		**1**	**<0.1**	**<0.1**		**1**	**<0.1**	**0.7**

3.2. Polymer analysis

Plastic polymers ingested by sea turtles in this study were, in descending order of importance: polyethylene (PE), polyamide (PA), polyvinyl vinyl acetate (EVA), polyurethane (PUR), polypropylene (PP), poly acrylonitrile butadiene styrene (ABS) and polystyrene (PS) (Table 4). Most of the polymers analysed belonged to the green turtle samples, as this species presented the highest variation and abundance of ingested plastic fragments. Although PS was common in green turtle GTIs (41.0%), few samples were composed of this polymer because could not be completely dried and analysed. Among the polymers identified, PE, EVA, PP and ABS, are less dense than the seawater (~1.025 g cm^{-3}; Table 4) and PA, PU and PS are denser. Signs of degradation were identified in 108 samples (84.4%), demonstrating that most items ingested by sea turtles were in the marine environment for at least six months prior to ingestion and could have originated from distant areas, transported by ocean current (Kessler, 2014; Van Sebille et al., 2015).

Table 4. Polymeric composition of plastic fragments ingested by sea turtles at southern Brazil between 2013 and 2017. N = number of fragments, density of polymers, and FO% = frequency of occurrence of each polymer.

Polymer	**N**	**Density of polymers (g cm^{-3})**	**Floats/sinks**	**FO%**
Polyethylene (PE)	60	0.91 – 0.95	Floats	46.9
Polyamide (PA)	22	1.04 – 1.13	Sinks	17.2
Ethyl vinyl acetate (EVA)	18	0.92 – 1.06	Floats/sinks	14.1
Polyurethane (PUR)	14	1.20 – 1.24	Sinks	10.9
Polypropylene (PP)	8	0.83 – 1.00	Floats	6.3
Acrylonitrile butadiene styrene (ABS)	5	0.92 – 1.00	Floats	3.9
Polystyrene (PS)	1	1.04 – 1.05	Floats/sinks	0.8
Total	128	-		100.0

3.3. PML ingestion by green turtles over time

Mean mass of PML ingested by green turtles over the evaluated period ranged from 0.5 (1997) to 28.7 g (2010), with significant difference among all evaluated years (χ^2= 81.4; p <0.001) (Fig. 2). The FO% of PML intake by green turtles was high since 2006, with the exception of 2013. Over the years, FO% ranged from 25.0 (2013) to 100% (2006, 2007, 2015, 2017), being equal to or above 70.0% in most years. In 2007, 2010, 2015 and 2017, PML ingestion was higher than the other years in terms of mean mass of litter ingested per individual. In 1997, 2012 and 2013 a lower intake of PML was observed in mass and FO% (Fig. 2).

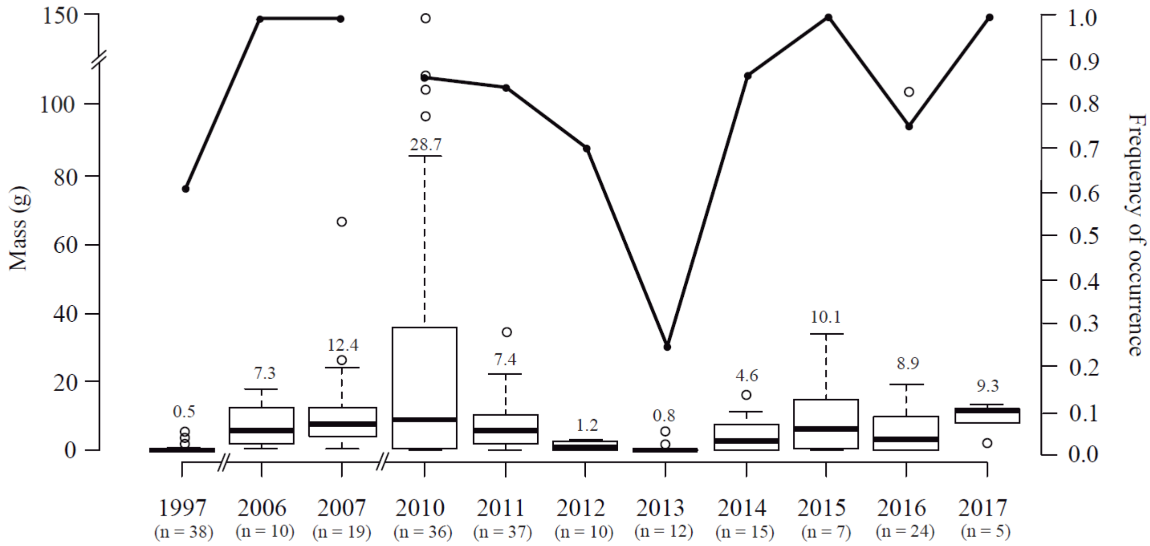

Fig. 2. Variation over time in ingestion of PML by green turtles *Chelonia mydas* in southern Brazil. Boxplots show the total mass of PML found in turtles by year and solid lines show the frequency of occurrence of ingestion over all years analysed. Numbers above whiskers represent mean mass among individuals; dashed lines represent median mass; white circles represent the outliers.

3.4. Influence of habitat and feeding strategy

In terms of habitat, PML ingestion by sea turtles was high for individuals classified as neritic and oceanic (Fig. 3a). Individuals classified as neritic had an ingestion rate of PML of 57.7%, and those classified as oceanic had 50.0%. When comparing PML ingestion between neritic and oceanic loggerheads, the FO% of PML intake was significantly higher (t = -6.4, p = 0.001) for oceanic turtles (FO% = 91.4) than neritic turtles (FO% = 29.2). Similarly, PML volume was significantly higher (χ^2= 47.6; p = 0.037) in oceanic turtles (mean = 7.2 ± 9.4 cm^3) than in neritic ones (mean 1.6 ± 5.4 cm^3) (Fig. 4). The ingestion of litter according to feeding strategy of all species was higher for omnivorous individuals (80.0%), more than twice that observed for the individuals classified as carnivores (Fig. 3b).

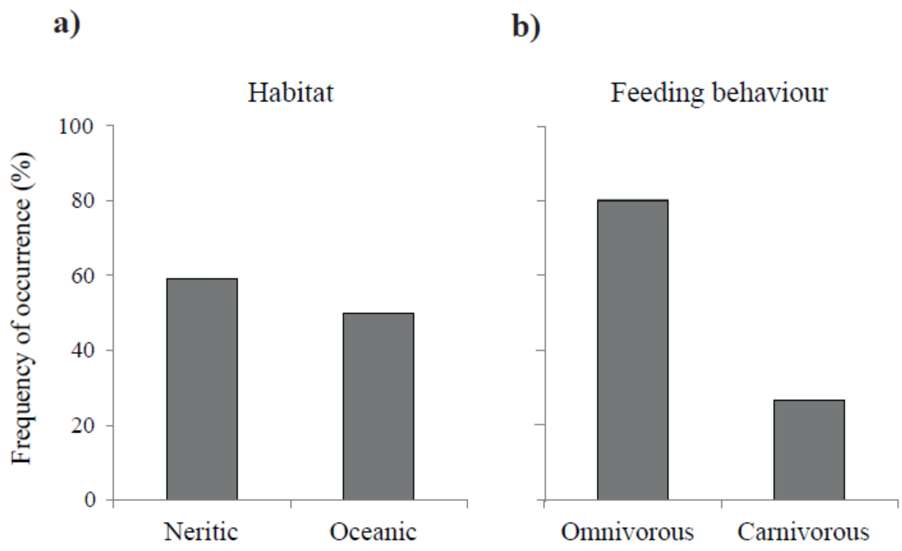

Fig. 3. Frequency of occurrence (%) of ingestion of plastic marine litter per (a) habitat and (b) feeding behavior of sampled sea turtles at southern Brazil between 2013 and 2017.

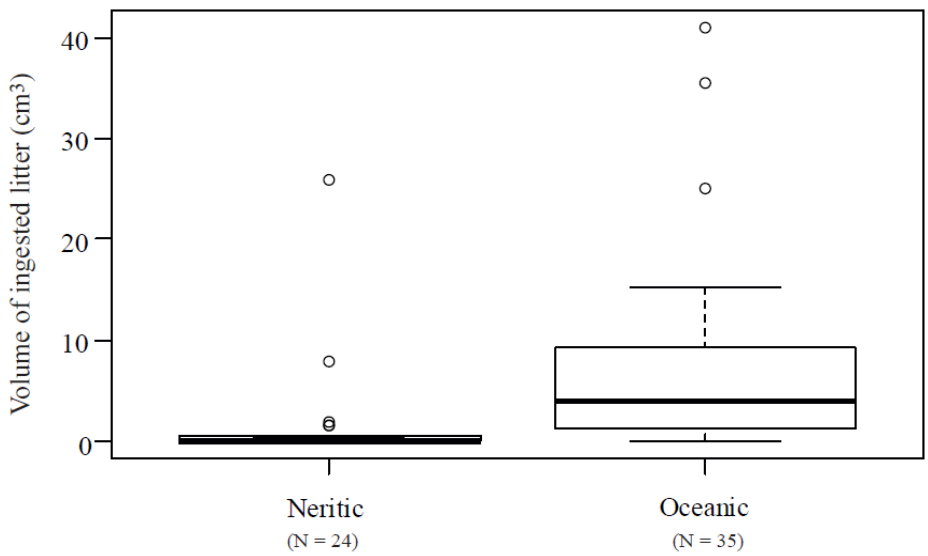

Fig. 4. Volume of PML ingested by loggerhead turtles *Caretta caretta* turtles at southern Brazil between 2013 and 2017 according to habitat. Boxplots represent the volume of debris ingested by turtles in neritic and oceanic habitats; line within boxplot represents the median; circles represent outliers.

3.5. Sample size, litter variability and ingestion by turtle size

For green turtles, the number of sampled individuals (n = 48), as well as the number of individuals who ingested PML (n = 39), was high. The accumulated curve for this species demonstrated that types of litter found represented most of the items that could be ingested by this species, since the curve approached the asymptote (Supplementary material Figure S3). For the loggerhead turtle (n = 24), there was a lower occurrence of PML intake (n = 7), as well as fewer types of ingested items. The curve for this species represented some of the types of PML that this species could ingest, but the variability of items could be larger if more samples were evaluated for this species (Supplementary material Figure S3).

The relationship between total number of ingested fragments and turtle size (CCL) was different for greens and loggerheads (Fig. 5). For the green turtle, the total amount of ingested items did not significantly correlated with animal size (Rho = -0.057; p = 0.08), with varying amounts of intake being observed among the evaluated CCL range (Fig. 5a). However, a significant negative correlation was observed between the number of ingested items and the size of loggerhead turtles (Rho = -0.716; p < 0.001, Fig. 5b). For this species, it was observed that 70% of the individuals with CCL < 70 cm (n = 7) ingested at least one fragment, whereas in turtles with CCL ≥ 70 cm (n = 14) there was no ingestion.

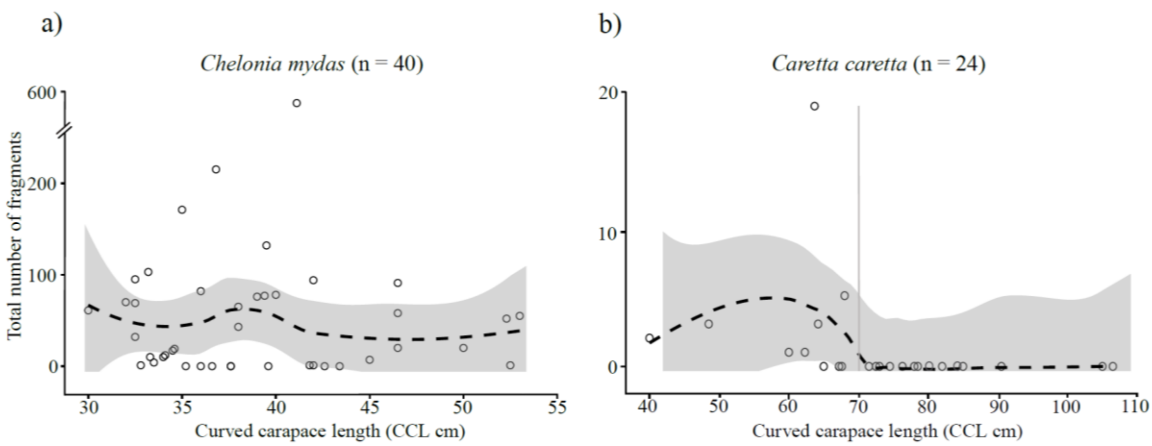

Fig. 5. Correlation between total number of ingested PML fragments and turtle Curved Carapace Length (CCL cm) for (a) green *Chelonia mydas* and (b) loggerhead *Caretta caretta* sea turtles.

3.6. Importance of PML type and color for ingestion

For the green turtle, the GLM showed that there was significant interaction ($p < 0.001$; $R^2 = 0.064$) between the type and color of PML ingested. Therefore, the type and color of the fragments were evaluated together for FO%, %N, %M and %PSIRI, resulting in 39 type/color combinations for ingested items (ations considered important in the ingestion analysis were those that presented %PSIRI ≥ 2.56 (cutoff factor = 100/S), which resulted in nine combinations (Fig. 6a and 6b). Based on the FO%, %N, %M and %PSIRI of the type and color categories ingested by the green turtle, three combinations of PML stood out: Supplementary Material - Table S1). The item combinflexible transparent fragments, white flexible fragments and white hard fragments. When each type and color category was evaluated, the transparent fragments represented the highest frequencies in the total of ingested items (FO% = 89.74, % N = 25.1% and % M = 14.8; Fig. 6a) and highest prey-specific index for the mean of each individual in the total of ingested items (%PSIRI = 28.9, Fig. 6b). Among the other categories, white hard fragments had higher numbers (%N = 17.4) and mass (%M = 23.0), and white flexible fragments had a smaller number (%N = 7.8) and mass (%M = 3.1) but higher frequency (FO% = 79.5) than the rigid fragments (FO% = 66.7) in the total number of items ingested by turtles (Fig. 6a). Thus, when the prey-specific index was calculated, white hard fragments (%PSIRI = 11.5) were almost equal to the white flexible fragments (%PSIRI = 12.4) ingestion (Fig. 6b).

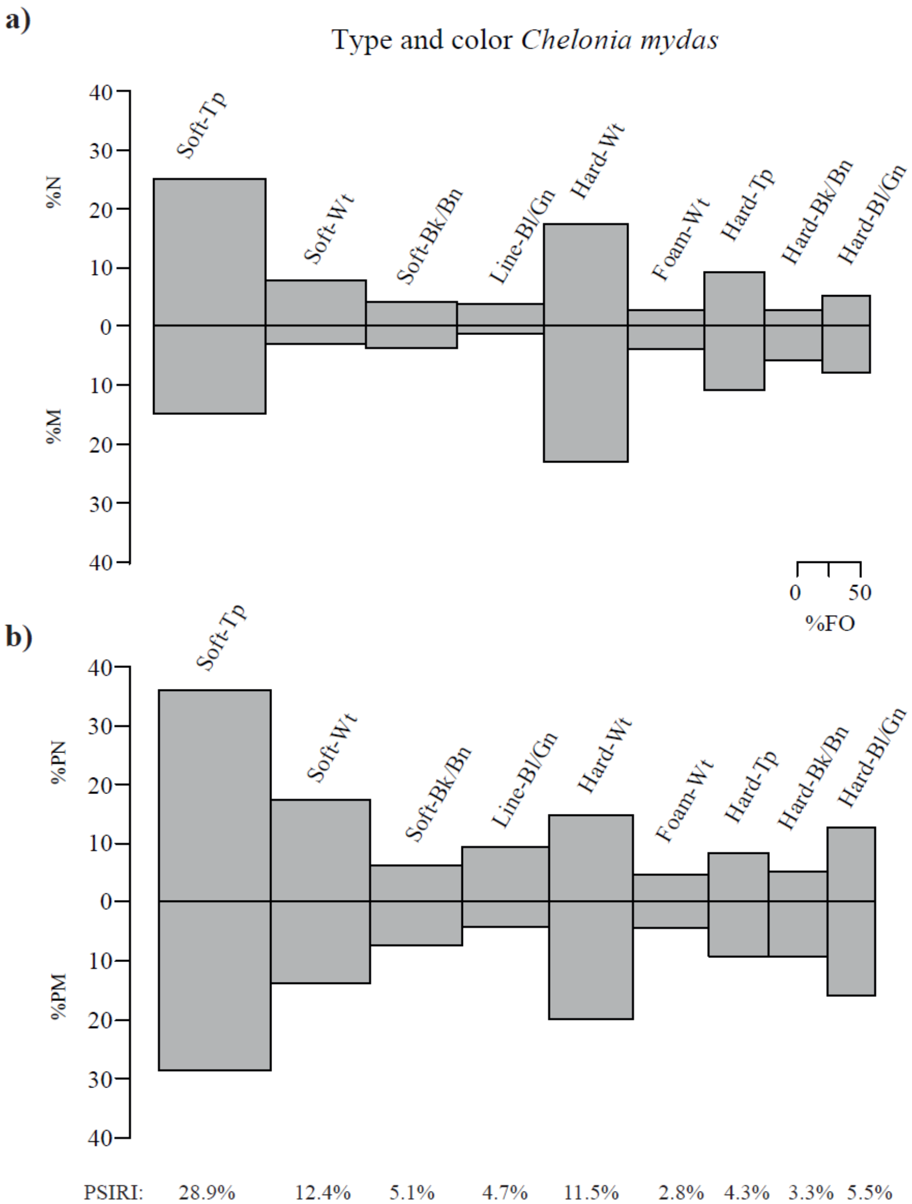

Fig. 6. (a) Frequency of occurrence (FO%), numerical percentage (%N) and mass percentage (%M) and (b) Prey-specific Index of Relative Importance (%PSIRI) of ingested fragments in terms of plastic type and color interaction for green turtles *Chelonia mydas*. Bk/Bn = black/brown, Bl/Gn = blue/green, Rd/Pk/Or = red/pink/orange, Tp = transparent, Wt = white.

For the loggerhead turtle, there was no interaction between the characteristics of each fragment (p = 0.78), and therefore the fragments were evaluated separately for type (R^2 = 0.02) and color (R^2 = 0.03) in terms of FO%, %N, %M and %PSIRI (Fig. 7a and 7b; Fig. 8a and 8b; Supplementary material Table S2). Six types and five colors of items ingested by this species were found. In terms of number and mass, the types most ingested by loggerhead turtles were hard fragments (%N = 23.5, %M = 47.3) while flexible fragments had smaller number and mass (%N = 8.8, M% = 0.7), but the FO% of both was the same (42.9%) (Fig. 7a). Lines were much more numerous (41.2%) and rubber had higher mass (40.8%) in the total items ingested by this species. When we evaluated the prey-specific index was observed high importance of hard fragments (% PSIRI = 29.6), followed by XPS/foam (18.3) and flexible fragments (17.7) (Fig. 7b). Rubber and paper presented %PSIRI of 100%, but only because they were the only types of items within the respective GITs (Fig. 7b). Regarding color, white items presented the highest numerical values (%N = 26.6), mass (%M = 57.8) and frequency (%FO = 57.1), while black/brown items had high frequency (FO% = 42.9) but lower percentages in number (%N = 17.6) and mass (%M = 2.9) (Fig. 8a). Ingested items in the blue/green category presented high number (32.4%) and those in the red/pink/orange category, high mass (35.7%). When evaluating the importance of colors by the prey-specific index, the highest importance was white (%PSIRI = 40.1) and black/brown (% PSIRI = 29.4) (Fig. 8b). Similar to what was found analyzing the %PSIRI by type for some individuals, the red/pink/orange category was the only one found in one GIT and therefore represented 100% in number and mass.

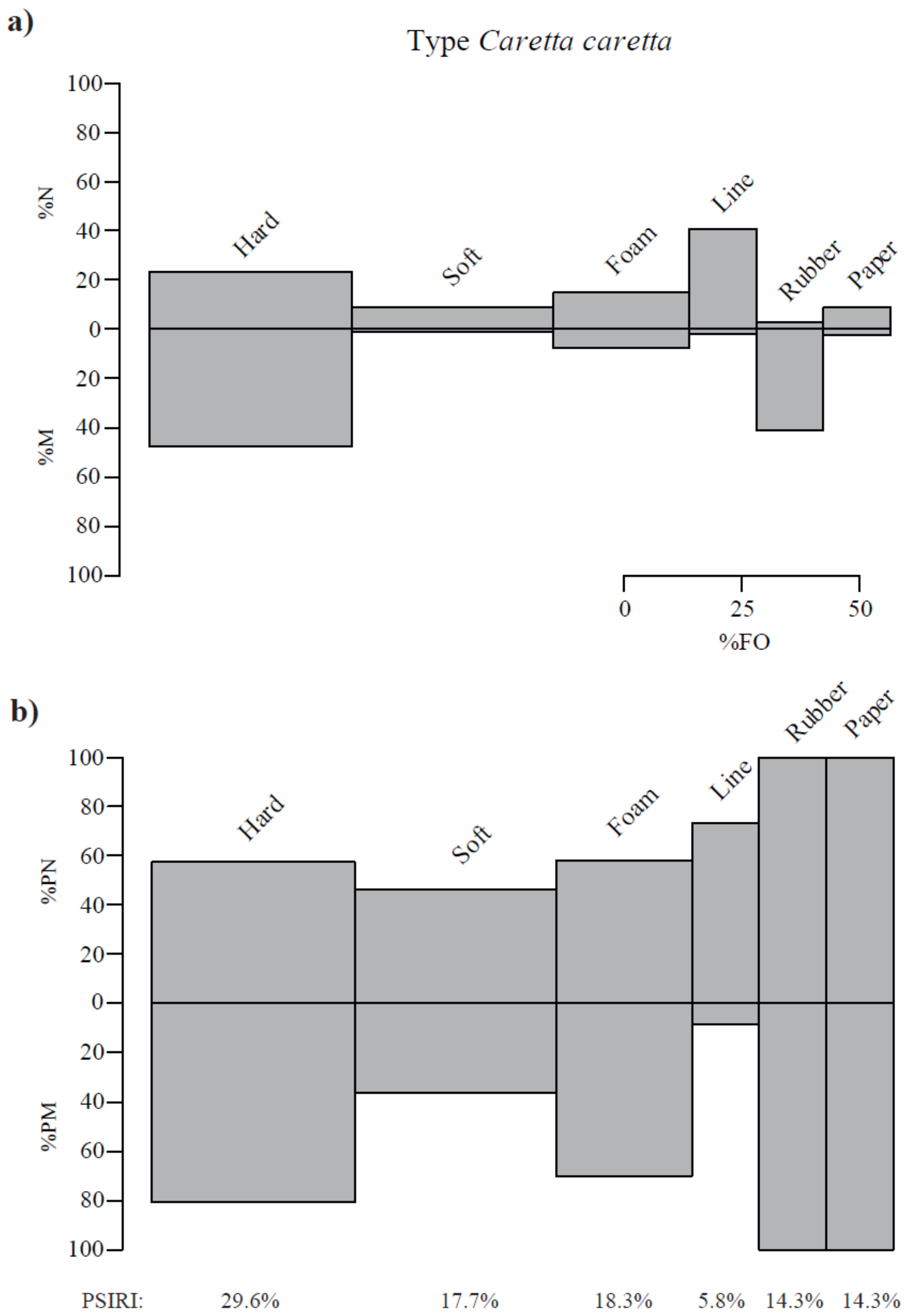

Fig. 7. (a) Frequency of occurrence (FO%), numerical percentage (%N) and mass percentage (%M) and (b) Prey-specific Index of Relative Importance (%PSIRI) of ingested fragments in terms of type for loggerhead turtles (*Caretta caretta*). Bk/Bn = black/brown, Bl/Gn = blue/green, Rd/Pk/Or = red/pink/orange, Tp = transparent, Wt = white.

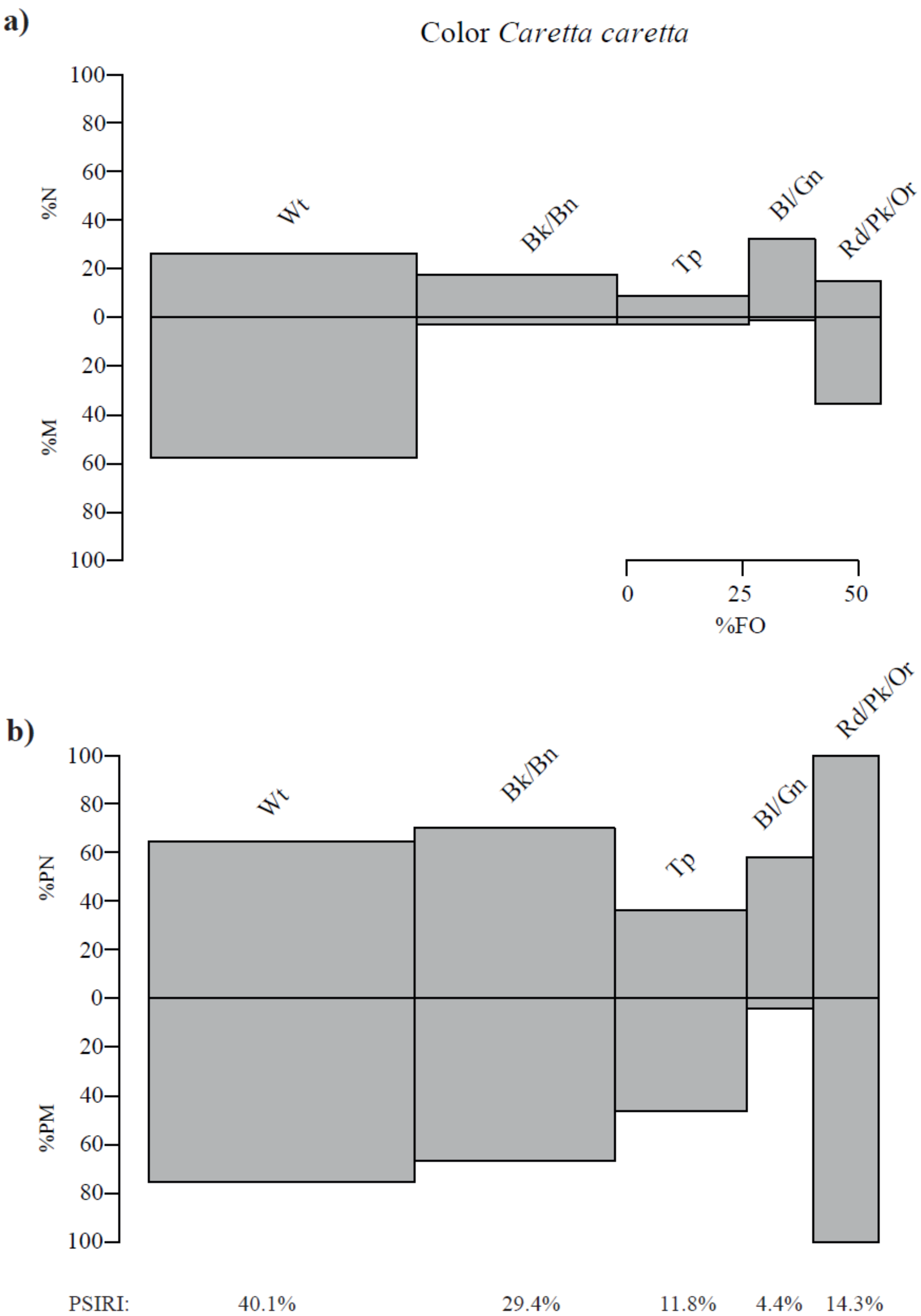

Fig. 8. (a) Frequency of occurrence (FO%), numerical percentage (%N) and mass percentage (%M) and (b) Prey-specific index of relative importance (%PSIRI) of ingested fragments in terms of color for loggerhead turtles (*Caretta caretta*). Bk/Bn = black/brown, Bl/Gn = blue/green, Rd/Pk/Or = red/pink/orange, Tp = transparent, Wt = white.

4. Discussion

4.1. Amount, frequencies and characteristics of ingested PML

In this study we evaluated PML ingestion by the five species of sea turtles that occur in southern Brazil and observed that more than half (57.0%) of the evaluated animals ingested litter, in five species. This high frequency of PML ingestion by sea turtles is in line with global records, confirming the seriousness and wide extent of this threat to these animals (Kühn et al., 2015). Evaluating PML ingestion for all species that occur at the studied region allowed us to identify differences in terms of the biological and ecological characteristics of animals, as well as the variety of types and colors of plastics, indicating differential ingestion according to habitat/feeding preferences and availability of plastics in the ocean. We demonstrate that plastic ingestion by sea turtles is a continuous problem at the region, greatly impacting green and loggerhead turtles and also affecting hawksbill, leatherback and olive sea turtles.

The highest occurrence of PML ingestion was observed for the green turtle (FO = 81.3%), demonstrating the impact suffered by juveniles of this species in southern Brazil. Previous studies at the region also reported high %FO ingestion values of: 60.5% (only esophagus and stomach evaluated; Bugoni et al., 2001), 100% (Tourinho et al., 2010) and 72.6% (Colferai et al., 2017). Along the Brazilian coast, juvenile and adult green turtles also present high frequencies of PML ingestion, with FO = 70 to 100%, demonstrating that this intake is common and widespread along the Brazilian coast (Santos et al., 2015). For the nearby Uruguayan coast, high values of PML intake were also found for juvenile individuals, varying from 70% (Vélez-Rubio et al., 2018) to 90% (Carman et al., 2014). In the North Atlantic, reported litter ingestion for this species was 46.7% in the Gulf of Mexico (Plotkin and Amos, 1990) and 56% in Florida (Bjorndal et al., 2004), in individuals analysed in the 1990s. In the North Pacific, between 90 and 100% of sampled green turtles have been shown to ingest PML (Wedemeyer-Strombel et al., 2015; Fukuoka et al., 2016; Clukey et al., 2017). Santos et al. (2015) verified that mortality due to the ingestion of PML in juvenile green turtles stranded along the Brazilian coast was 10.7%, and the authors indicate that the potential mortality may be even higher reaching 39.4% of stranded individuals, due to sublethal effects. The high ingestion of PML by green turtles worldwide is a serious threat to the survival of this species, especially during the juvenile phase when they feed at shallow and coastal regions where PML availability is increasing, due to increased discards.

In loggerhead individuals (mostly juveniles), a low frequency of PML (FO = 29.2%) was observed. For the region, was recorded previously a frequency of ingestion of 10% of the individuals with esophagus and stomach evaluated (Bugoni et al., 2001). The frequency of ingestion found here is similar to loggerheads sampled in the Mediterranean at the Adriatic Sea (Slovenia and Croatia) (35.2%; Lazar and Gracan, 2011) and Lampedusa Island and Silesia (Italy) (35.4%; Casale et al., 2016), but smaller than in loggerhead turtles sampled at the coast of Portugal (59%; Nicolau et al., 2016). However, higher frequencies of intake for this species have been observed elsewhere: 84.6% in Japan (Fukuoka et al., 2016), 80% in the center of the North Pacific Ocean (Clukey et al., 2017) and 83% in the subtropical gyre region of the North Atlantic, in the Azores region (Pham et al., 2017). Although the frequency of ingestion at the study region was lower than other previous regions, ingestion was observed as already recorded for southern Brazil. Even the PML intake by this species was lower than in some other sites, interaction with PML occurred in almost 30% of the animals, representing a threat to the species. In addition, %FO of PML ingestion by the loggerhead turtle may be underestimated, since this species has a broad GTI (Bugoni et al., 2001) that could favor PML defecation and removal (Hoarau et al., 2014).

Only two juvenile hawksbill individuals were collected during this study, since this species uses mostly tropical areas and beaches in Brazilian northeastern coast; PML ingestion occurred in one of these individuals (i.e. 50.0%). At northeast Brazil, a high frequency of PML intake was recorded respectively for juveniles (FO of 77.8%; Macedo et al., 2011) and juveniles and adults of this species (41.7%; Poli et al., 2014). Similarly, in the North Atlantic, a high amount of litter ingestion has been reported for this species, occurring in 87.5% of the individuals (n = 8) analysed in the 1990s (Plotkin and Amos, 1990). This indicates that PML ingestion is frequent in this species, especially in the juvenile stage when they present opportunistic and generalist feeding strategies, similarly to the green turtle (Schuyler et al., 2012).

Of the four leatherback turtles analysed in this study, one ingested PML (FO = 25.0%). In the same study region, Bugoni et al. (2001) analysed the esophagus and stomach of two individuals, recording litter ingestion in one. This species preferentially uses the oceanic habitat throughout its life (Almeida et al., 2011b) and does not usually strand on Brazilian beaches; this occurs more frequently in the southern region (Barata et al., 2004; Monteiro et al., 2016). However, there is a paucity of data on the ingestion of litter in leatherback turtles in this region, as well as elsewhere in the world (Mrosovsky et al., 2000; Duguy et al., 2009). Mrosovsky et al. (2009), when analyzing adult leatherback turtle data collected between 1885 and 2007 in various parts of the world, observed that PML intake occurred in 34% of the individuals (n = 408) and that there was an increase in intake between 1960 and the early 2000s. Since leatherback turtles present a specialized feeding strategy (mainly gelatinous organisms), the ingestion of litter may be especially related to the availability of some types of PML (e.g. bags, clear flexible fragments) in the foraging environments.

Olive ridley turtles presented the lowest PML intake among the species analysed in this work (FO = 12.5%), different from the few existing studies for the species, in which reported %FO have been high. For example, in north Brazil, the frequency of occurrence of PML ingestion in three adults/subadults was 100% (Mascarenhas et al., 2004; Poli et al., 2014). High frequencies of PML ingestion were also observed in turtles sampled in the center of the North Pacific Ocean (FO = 82%; Wedemeyer-Strombel et al., 2015 and FO = 100%; Clukey et al., 2017). Thus, PML represents a threat to some populations, although it does not seem to be a large issue in southern Brazil considering the low %FO and the amount of litter ingested by the analysed adult and subadult animals. This can be explained by the fact that the olive ridley presents a more specialized foraging strategy in these life stages, feeding on active prey such as fish, crustaceans and squids (Di Beneditto et al., 2015).

GIT obstruction and fecaloma formation were observed in this study for green and loggerhead turtles. These detrimental effects indicate that death possibly occurred due to ingestion of PML, which even in low amounts can have negative consequences (e.g. lesions to TGI, blocking stool passage) in animals (Bjorndal et al., 1994). Santos et al. (2015) observed that only 0.5 g of litter was sufficient to cause lethality by blocking the GIT in green turtles, suggesting this value as the critical mass to generate death, and that for most individuals the mass of litter was greater than that. In our study, obstruction and formation of fecalomas in green turtles occurred in approximately one third of the individuals analysed (n = 15) and mass was over 8 g of material, indicating that individuals may have died due to ingestion of PML. A single loggerhead presented an obstruction at the end of the GIT, and had ingested 11.1 g of litter. This individual was one of the smallest analysed in this study (CCL = 48.5 cm), demonstrating that ingestion may affect smaller individuals of this species as they are more prone to obstruction and formation of fecalomas. Casale et al. (2016), studying juvenile and adult loggerheads from the Mediterranean, reported that the ingestion of PML did not lead to the death of animals. However, in post-hatchlings of this species sampled in southern Africa, PML ingestion was held responsible for the death of 11 individuals from lethal damage and contributed to the death of five others due to sub-lethal lesions (Ryan et al., 2016).

4.2. Characteristics of ingested PML

PML can be found in marine and coastal ecosystems in different forms and compositions, and plastics make up the large majority of waste in these environments (Derraik, 2002). Thus, plastic fragments were the most frequently and abundantly ingested litter by sea turtles in this study, as well as worldwide (Schuyler et al., 2014a; 2016; Santos et al., 2015; Clukey et al., 2017). Hard and flexible fragments, packaging, bags and lines were the main items found in the GITs of the animals analysed. These types of plastics are the most commonly ingested items by sea turtles globally (Schuyler et al., 2014a; Clukey et al., 2017; Colferai et al., 2017; Pham et al., 2017; Vélez-Rubio et al., 2018). Other types of plastics, such as XPS, ribbons, entangled wires and fibers, also occur in the GITs of green turtles. The high ingestion rates of these types of plastic items may be associated with a greater availability of these materials in the environment, mainly due to their large production and inappropriate disposal on land or at sea, high durability, or characteristics in shape, material or color that lead to a greater likelihood of ingestion (Schuyler et al., 2012; 2014a).

Green turtles ingested a large variety of PML types, presenting the highest values of ingestion in both FO and in mass and number of items. The same has been observed in the center of the North Pacific Ocean, where green turtles ingested a greater amount of PML than olive ridleys, loggerheads and leatherbacks sampled at the same area, possibly due to their smaller size and ingestion of flexible plastics that move more slowly along the GIT (Clukey et al., 2017). In the current study, the plastic items most ingested by green turtles were packaging, fishing lines and rigid fragments. In addition to plastics, non-plastic items (i.e. rubber, wood and fabric) were also ingested, with party balloons (rubber) being the most frequent. Schuyler et al. (2012) observed that green and hawksbill turtles also ingested rubber balloons during their feeding, and attribute this to the similarity of the balloons with their prey (e.g. jellyfish, squid, algae), since this material is not frequent in the marine environment. In addition, the authors suggest that buoyancy and high malleability of balloons may contribute to the easy detection and ingestion by sea turtles.

Loggerhead turtles ingested more frequently hard plastic fragments and fishing lines, as observed for individuals sampled in the Pacific Ocean (Wedemeyer-Strombel et al., 2015) and the North Atlantic (Pham et al., 2017). The ingestion of hard fragments by this species may be related to its benthic feeding habit (Bjorndal et al., 1997; Di Beneditto et al., 2015), since fragments with a higher density than seawater are deposited on the seabed. The high intake of fishing lines may be due to interactions with fishing activities in the region (e.g. ingesting chunks of ropes and discarded lines, biting nets), since there is an overlap between loggerhead foraging sites and fishing grounds (mainly trawling fisheries) in the inner continental shelf on southern Brazil (Monteiro et al., 2016). The hawksbill, leatherback and olive ridley individuals analysed in this study only ingested flexible fragments in low quantities (four items for all individuals). Due to the low number of sampled individuals, it was not possible to determine an ingestion pattern at the region. Despite the small sample size, the present study is one of the few to have evaluated PML ingestion by these three species along the Brazilian coast, generating information that contributes to a better understanding of the interaction between sea turtle species and PML.

4.3. Polymer composition

The plastic polymers most commonly found in the ocean are PE (high and low density) and PP, followed by polyvinyl chloride (PVC), PS, polyethylene terephthalate (PET) and PUR (Morét-Ferguson et al., 2010; Ivar do Sul and Costa, 2014; UNEP, 2016). PE and PP represent the majority of the world's plastic production and consequently waste flow to the oceans (Jambeck et al., 2015; Plastics Europe, 2017). These two types of synthetic polymers float in the oceans, while others are denser than seawater (e.g. PVC, PA) and sink; however, even denser polymers can float over time due to degradation that reduces their molecular weight (Andrady, 2011). In the center of Pacific Ocean, the main ingested polymers by turtles captured were PE, PP, mixtures of PE and PP, PS, PVC and PA (Jung et al., 2018), which are present in most items found in the sea.

In our study, most fragments ingested by sea turtles were identified as PE, which represented 46.9% of the analysed items, followed by PA (17.2%) and EVA (14.1%). PE can be of low density (e.g. plastic films, bags) or high density (PEHD, e.g. pots, toys) (Plastics Europe, 2017), and was identified mainly in flexible fragments as bags and packaging, but also in fragments of disposable cups and hard fragments. PA is commonly used in fisheries (e.g. lines, ropes) and in the textile industry, but can be used in several plastic items, being present in flexible and hard fragments observed in this study. EVA, in addition to being identified in flexible and hard fragments, was present in samples of foamed plastics such as sponges. Despite being one of the most produced polymers in the world (Plastics Europe, 2017), PP presented low occurrence in this study (6.3%). The polymer identified, along with the type and color of the items may have determined this intake. Understanding the predominant polymers found in habitats and ingested by marine organisms can guide conservation efforts, including changes in recycling strategies, waste management direction, and new approaches to polymer production (Ryan et al., 2009).

4.4. PML ingestion by green turtles over time

There was no clear pattern of PML ingestion by the green turtle over the three analysed time periods (1997, 2006-2007, 2010-2017). However, it is evident that in the area covered by this study, green turtles are under constant threat from PML ingestion, presenting ingestion FO equal to or above 70% for most analysed years. Lower %FO was observed in 2013 (<50%). In terms of mass of ingested PML, 1997, 2012 and 2013 presented low intake mass (mean of 0.5, 1.2 and 0.8 g per individual, respectively) and in 2010 the highest mean PML ingestion mass (28.7 g) was recorded for one animal; over the remaining years, mean and median mass of ingested PML had similar values. The high mass observed in 2010 was likely a consequence of recording the wet mass of the material in this year (Ruzzene, 2011), and not the dry mass as in other periods. Even so, the %FO of 2010 (86%) shows a high ingestion rate. On the coast of Uruguay, it was also observed that the %FO of PML ingestion by juvenile green turtles remained constant over the years, but that mass and volume were higher between 2009-2013 than between 2005-2007 (Vélez-Rubio et al., 2018), indicating an increase in ingested amount over the years. The constant and abundant ingestion of PML by this species, as well as the apparent increase in the amount of PML ingestion is worrisome, as it results in a greater probability of generating sub-lethal and lethal effects for green turtles at these regions.

Comparison of PML ingestion over time was challenging due to the lack of common data among the previously performed studies, which made it impossible to carry out more detailed analyses. The use of several variables and characteristics of PML ingestion allows a greater refinement of records and comparisons between regions and periods (Ivar do Sul and Costa, 2007). Although the frequency of occurrence of litter ingestion is an important factor in these evaluations, quantification measurements (i.e. number, mass, volume) and the characterization of ingested items (i.e. material, type, color) allow a more complete analysis of the impacts and patterns of PML ingestion. Since the characteristics of PML can influence their ingestion by sea turtles (Casale et al., 2016), they should be consistently recorded and evaluated. Thus, we emphasize the importance of increasing the quantity and standardizing the obtained information, with records of at least the following variables: presence/absence, number of items, mass and volume of each item, material, type, color and flexibility, and identification of obstructions and/or formation of fecalomas.

4.5. Influence of habitat and feeding strategy in PML ingestion

The probability of interactions between sea turtles and PML is related to the feeding ecology and habitat occupied by different turtle species, factors that depend on the geographic region, population and/or life stage in which individuals are found (Schuyler et al., 2014a). In relation to the occupied habitat, sea turtles classified as oceanic and neritic showed both high PML intakes. In the oceanic environment, sea turtles generally feed at the air-water interface or first meters of depth, preferably close to oceanic gyres and front/convergence zones, where there is a high concentration of food (Polovina et al., 2004; Schuyler et al., 2012; Wedemeyer-Strombel et al., 2015). At these regions, floating PMLs tends to accumulate, increasing the likelihood of ingestion by marine biota (Schuyler et al., 2012; Nelms et al., 2015). Juveniles in the oceanic environment may be at high risk of PML intake also due to the lower selectivity in diet at this stage of the life cycle; additionally, ingestion may have greater impact due to the smaller size of the GIT (Schuyler et al., 2012). At the center of the North Pacific Ocean, sampled juvenile and adult green, loggerhead and olive ridley turtles presented high frequencies of PML ingestion (Wedemeyer-Strombel et al., 2015; Clukey et al., 2017). At the eastern coast of Australia (Queensland), oceanic green and hawksbill turtles presented higher litter ingestion when compared to coastal turtles feeding on the seafloor (Schuyler et al., 2012). This was also observed for loggerhead turtles in the Mediterranean, at Lampedusa Island and Silesia (Italy) (Casale et al., 2016). Although sea turtles in the oceanic habitat are primarily juveniles of smaller size, there are certain populations in which individuals use oceanic environments even when subadults or adults (Mansfield and Putman, 2013; Petitet et al., 2015). In this way, it should be considered that there are variations in diet and, consequently, in PML ingestion between populations, and the comparisons made here are based on the general pattern of the life cycle of the considered species.

In the neritic environment, the risk of PML ingestion can also be high due to the proximity to land and urban centers, which are the main source of litter that reaches the oceans through rivers, sewers, estuaries, wind and coastal discards (Sheavly and Register, 2007; Ryan, 2014; Lebreton et al., 2017). The constant entry of these materials into coastal areas can generate accumulation and increase the availability for ingestion by marine animals that feed at these regions. Santos et al. (2015) found a high risk of ingestion in estuarine areas and close to highly urbanized areas, corroborating the hypothesis that the availability of litter can lead to high ingestion by turtles, as well as by other groups of animals (Schuyler et al. 2014a; 2016). Thus, although the higher frequencies of PML intake were observed for turtles in the oceanic habitat, the neritic environment may present similar ingestion values (as observed in this study), depending on the degree of pollution of the occupied area.

The life cycle pattern of the loggerhead turtle is generally described as oceanic-neritic, in which hatchlings disperse to the oceanic environment to develop, and recruit to the neritic environment after becoming larger juveniles (between 46 and 64 cm) (Bolten, 2003). However, neritic juveniles and adults of this species can change their habitat use according to resource availability and habitat selection plasticity (Hatase et al., 2008; Mansfield et al., 2009; Mansfield and Putman, 2013). In this study, turtles classified as oceanic had higher occurrence and volume of ingested PML than neritic ones, as observed in Mediterranean loggerheads (Casale et al., 2016). Although the data used in this analysis are not contemporaneous, have shown that the intake is quite different between the habitat used. Pham et al. (2017) point out that in the North Atlantic subtropical gyre loggerhead turtles are more prone to PML ingestion, and suggest that the accumulation of floating litter along with the species' feeding ecology in the oceanic environment may favor PML ingestion (Polovina et al., 2004; Nelms et al., 2015; Schyuler et al., 2015). Despite the greater ingestion by oceanic turtles, the interaction of neritic animals with litter is also concerning, and the investigation of PML ingestion in both environments is important. Moreover, the concentration of PML in some neritic regions may be higher than in oceanic regions (Ryan, 2014) and thus increase the possibility of ingestion.

In terms of feeding strategy, sea turtles classified as omnivorous presented a significantly higher PML intake than those classified as carnivorous. This higher intake by omnivorous individuals may be related to their more generalist diet and higher variety of prey items (Schuyler et al., 2014a). In northern Brazil (Paraíba state), it was observed that smaller juvenile turtles had a higher probability of ingesting PML than the larger ones, likely due to more generalist diet (Poli et al., 2014). In southern Brazil, green turtles occur in the juvenile stage, and their omnivorous and varied feeding behavior can lead to a greater interaction with PML (Bugoni et al., 2003; Tourinho et al., 2010). Juvenile hawksbill turtles are also omnivorous and generalist and may present high PML intake (Bjorndal, 1997; Schuyler et al., 2012), but due to the sporadic occurrence of the species at the study region, it was not possible to obtain an adequate number of samples to demonstrate this hypothesis.

As observed in this study, it has been reported that carnivorous species appear to be less susceptible to PML ingestion when compared to omnivores or preferentially herbivorous species, since they are more selective with their prey or because they have a larger capacity to egest PML fragments (Schuyler et al., 2014a). For example, loggerhead turtles in the neritic environment typically ingest active prey found in the benthic zone, making them less likely to ingest marine plastics (Bjorndal, 1997; Schuyler et al., 2016). Olive ridleys present omnivorous diets in some parts of the world (Schuyler et al., 2016), but in southeastern Brazil this species preys mainly on active animals (Di Beneditto and Awabdi, 2015), which may explain the low PML ingestion observed in the present study. The leatherback turtle is pelagic-carnivorous throughout its life, ingesting mainly gelatinous organisms (Saba, 2013), and being more susceptible to the ingestion of some types of litter such as plastic bags and flexible packaging when these are available in the oceans. Sea turtles can present inter-populational and individual differences in diet due to geographic region, life-cycle stage and individual food preferences, and PML ingestion should therefore be evaluated considering these factors (Casale et al., 2016).

4.6. Ingestion according to turtle size

In this study, no significant relationship was found between PML ingestion and green turtle size. For the same region, previous studies also did not observe variation in PML intake according to the size of the analysed individuals (Bugoni et al., 2001; Tourinho et al., 2010). The lack of relation between animal sizes the ingestion of litter can be attributed to the size range of the green turtle analysed at the region (30 to 60 cm CCL) – all of them juveniles, likely with similar food strategies and habitats. On the other hand, juvenile turtles that stranded along the coast of Uruguay presented a negative correlation between size and PML intake, with turtles with CCL smaller than 45 cm presenting a larger %FO of ingestion than those with CCL greater than 45 cm (Vélez-Rubio et al., 2018). In juvenile turtles caught in the North Pacific Ocean, the size of individuals was also negatively correlated with litter ingestion, possibly due to the recruitment of larger individuals into neritic habitats with less exposure to PML and greater diet specialization, as well as higher capacity of expelling litter (Wedemeyer-Strombel et al., 2015).

For the loggerhead turtle, a significant negative correlation was found between the sizes of the individuals analysed and PML ingestion, with a decrease in ingestion in animals over 70 cm CCL, which may be related to changes in diet after occupying preferentially the neritic region. In addition, because they have larger GITs and a high capacity of eliminating ingested litter (Bugoni et al., 2001; Hoarau et al., 2014), larger animals may be more easily eliminating PML. Similar results have been previously reported for sea turtles, including loggerheads, with decreased litter intake in larger-sized individuals sampled in the Gulf of Mexico (ingestion analysis in hatchlings until 40 cm, 40-80 cm, > 80 cm of CCL) (Plotkin and Amos, 1990); however, recent studies found no correlation between individual size and PML ingestion (Casale et al., 2008; Lazar and Gracan, 2011; Nicolau et al., 2016). Casale et al. (2016) verified greater PML ingestion in the size class between 40 and 60 cm of CCL. On the other hand, Campani et al. (2013) found a significant positive correlation between CCL and PML ingestion, but only four individuals smaller than 40 cm and 18 individuals larger than 40 cm were evaluated. Pham et al. (2017), evaluating loggerheads with CCL between 9 and 71 cm, observed that in addition to FO, the size of the animals is related to the size of the ingested PML items, verifying that the length of the ingested litter was positively related to the length of the animals. The lack of a clear pattern between litter ingestion and turtle size may be related to the variability of the sizes of the turtles sampled or differences in PML availability among the studied areas. Schuyler et al. (2012) state that biological and ecological factors, such as the size or stage of the life cycle, are important factors that influence the probability of PML intake, and that the ingested amounts also depend on the availability of litter in the environment.

4.7. Influence of PML type and color on ingestion

In this study, we verified that sea turtles ingested PML fragments differently according to the color and flexibility of the material. The green turtle mainly ingested a combination of flexible and light-colored items (transparent and white). This increased intake may be related to the shape and movement of these items in the water, attracting and confusing turtles with components of their diet (e.g. gelatinous animals), as suggested by Fukuoka et al. (2016). Schuyler et al. (2012) observed that green and hawksbill turtles from the neritic environment most commonly ingested white and clear flexible plastics, while oceanic turtles ingested more hard plastics and rubber. In the present study, in addition to flexible light-colored items, hard white fragments were also important in green turtle ingestion. This ingestion was also observed in juvenile green turtle from the Pacific, with ingestion of high amounts of flexible and hard white fragments (Clukey et al., 2017). On the other hand, Santos et al. (2016) found frequent ingestion of dark items (black and green) by green turtles. This intake was greater than the availability of plastics of these colors in the studied environments, suggesting that these dark items are more visible in the water than the light items. Despite the high frequency of clear and flexible plastic ingestion, in our study green turtles also ingested various other colors of fragments, including dark colors, which may be related to their opportunistic and omnivorous feeding and the availability of these items in the environment.

Loggerhead turtles ate mainly hard fragments, predominantly white, but also dark-colored (black and brown). In other studies for this species, greater occurrences of light-colored fragments, both hard and flexible, were also observed (Casale et al., 2016; Nicolau et al., 2016; Clukey et al., 2017; Pham et al., 2017). Our study showed that flexible fragments and foam were also important for the species, which along with hard low-density fragments, make up the floating material most commonly consumed by loggerhead turtles (e.g. Casale et al., 2008; Lazar and Gracan, 2011). Fukuoka et al. (2016) suggest that loggerhead turtles better distinguish artificial items (PML) from food items (natural prey) through their shape and movement, and therefore have lower frequencies of ingestion than green turtles. On the other hand, in the oceanic environment this species is more generalist and can consume a large range of items, leading to higher PML ingestion in this environment (Nicolau et al., 2016). In this study, loggerhead turtles had lower mass and number of PML items than in previous studies, but the type and color characteristics of the ingested items were similar.

Sea turtles are primarily visual predators (Fritsches and Warrant, 2013), using color and other visual cues to locate prey (Narazaki et al., 2013; Schuyler et al., 2014b). However, the preference of ingestion of certain PML colors is still controversial (Kühn et al., 2015), and as mentioned above, some authors affirm that the detectability and availability of litter in feeding grounds influence more its selection (Schmidt et al., 2004; Schuyler et al., 2012; Williard, 2013; Kühn et al., 2015). The materials most commonly ingested by sea turtles are single-use disposable plastics (Santos et al., 2015), which are available in high quantities in the environment in the form of flexible (e.g. derived from packages, bags) or hard fragments (e.g. derived from cups, pots). Sea turtles also frequently ingest fishing nets and their fragments, which are also among the main types of PML (Galgani et al., 2015). In this way, the ingestion of these materials may have greater occurrence due to their high availability in the marine environment (Kühn et al., 2015; Nicolau et al., 2016). The characterization of PML intake according to its detectability and availability in the environment can help us better understand the drivers behind litter ingestion, helping to predict its impact on different groups of animals and to mitigate these potential negative effects to marine life (Santos et al., 2016).

The availability of PML at surface waters and seafloor of the South Atlantic Ocean is largely unknown, with few studies being conducted and focused on microplastics (particles smaller than 5 mm; Ivar do Sul and Costa, 2014). A higher concentration of PML has been reported for the interior of the South Atlantic Subtropical Gyre, as observed for other oceans (Cózar et al., 2014; Ryan, 2014). At the southern Brazilian continental shelf, the Lagoa dos Patos and Río de La Plata can contribute to PML concentrations, since land-based sources represent a great contribution of litter to the oceans (Jambeck et al., 2015; Lebreton et al., 2017). Carman et al. (2014) describe the La Plata River as one of the most important sources of PML in the western South Atlantic. Thus, the assessment of PML diversity and concentrations in coastal and ocean waters, as well as the seafloor, is important to help understand the distribution and characteristics litter in the South Atlantic, crucial information for the prevention and mitigation of this type of pollution.

4.8. Final remarks

In this work, we evaluated PML ingestion by five sea turtle species in South Brazil, attempting to identify the characteristics and patterns of this ingestion. Understanding PML ingestion patterns by different sea turtle species is important to provide baseline information for the definition of public policies for the prevention of this problem, including: improving waste management; prohibition, reduction or taxation of the production of certain plastics; and changes in the use and consumption of disposable plastics. The high frequency of ingestion of single-use plastics and fishing gear emphasizes their great contribution to the global PML problem (Gregory, 2009). As global production and use of plastics continues to grow, marine plastics intake and their impacts on sea turtles and other marine animals will also increase (Schuyler et al., 2012); therefore, an adequate understanding of their effects on sea turtle populations is of high priority (Clukey et al., 2017).

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Supplementary Material

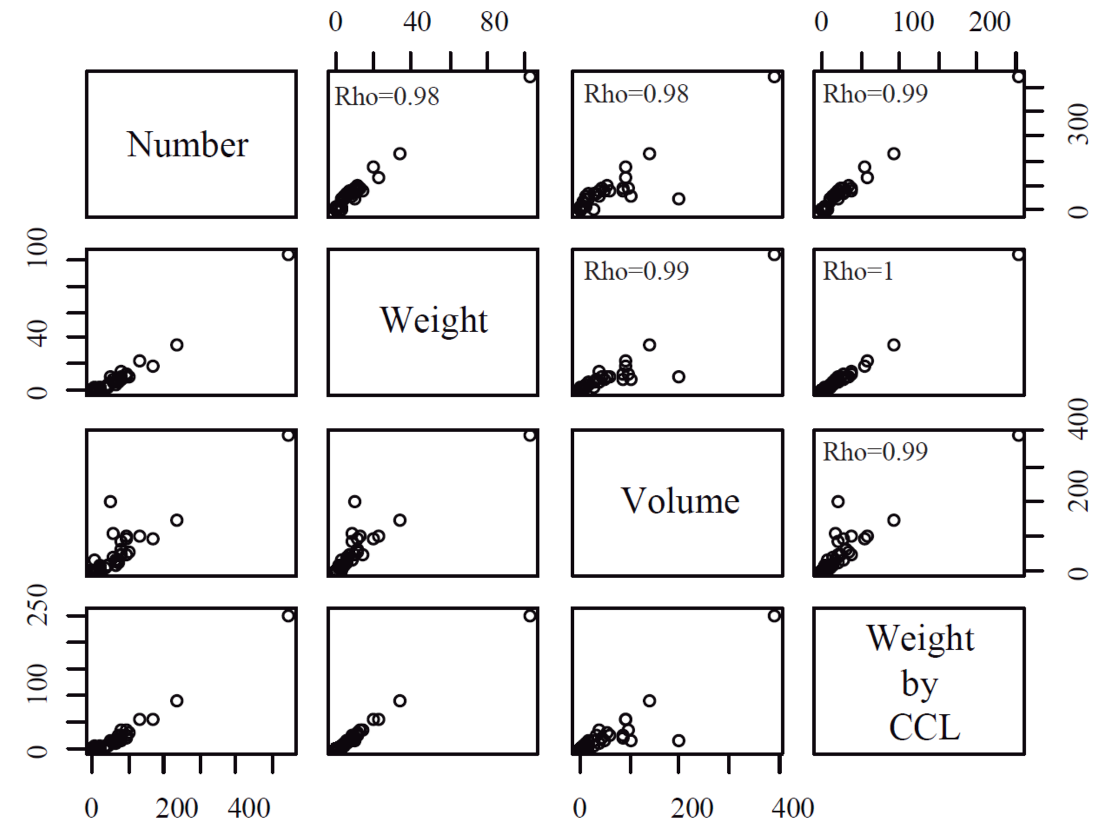

Figure S1. Correlations between number, mass and volume of ingested fragments, and mass of fragments by curved carapace length (CCL) of turtles.

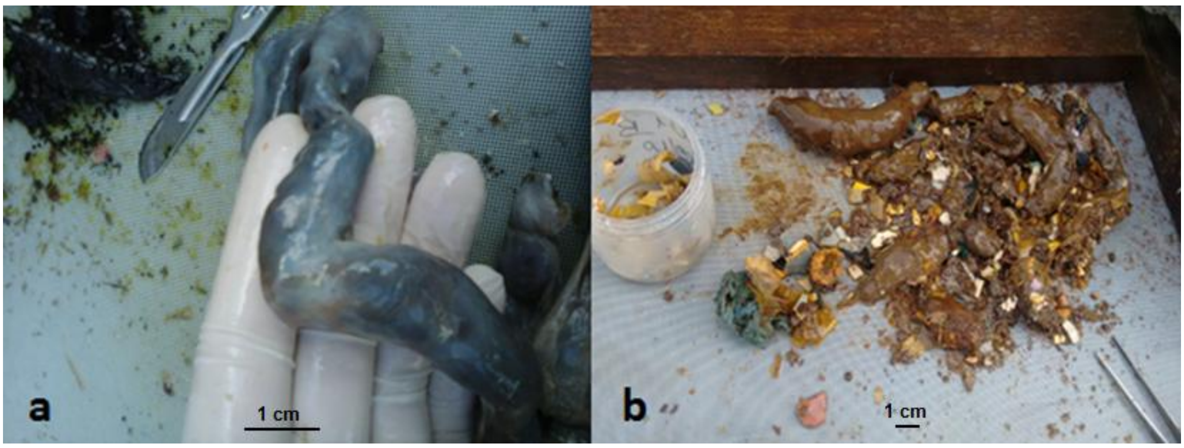

Figure S2. Marine litter in gastrointestinal tracts of green turtles *Chelonia mydas*: (a) obstruction with faecalomas; and (b) gastrointestinal content.

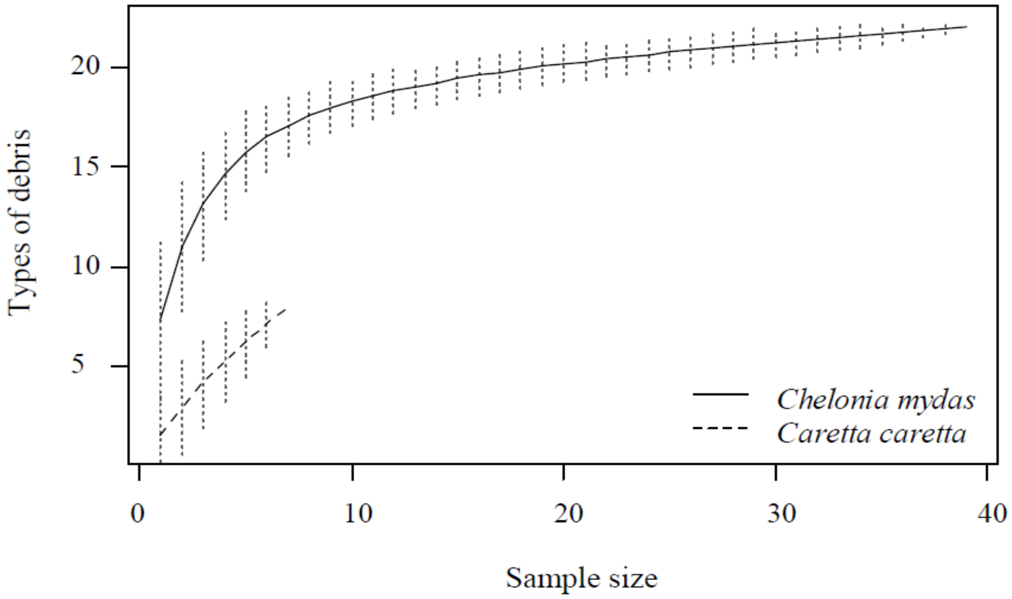

Figure S3. Species accumulation curve for types of plastic marine litter ingested by green *Chelonia mydas* and loggerhead *Caretta caretta* sea turtles at southern Brazil between 2013 and 2017.

Table S1. Type and color interactions for marine litter ingested by green turtles *Chelonia mydas*. FO% = frequency of occurrence, PN% = prey-specific numerical percentage, PM% = prey-specific mass percentage, PSIRI% = Prey-Specific Index of Relative Importance, %N = numerical percentage and %M = mass percentage.

Chelonia mydas						
Combinations of type and color	**FO%**	**PN%**	**PM%**	**PSIRI%**	**%N**	**%M**
Soft transparent	89.7	35.9	28.5	28.9	25.1	14.8
Soft white	79.5	17.5	13.8	12.4	7.8	3.1
Soft black/brown	74.4	6.2	7.4	5.1	4.2	3.6
Line blue/green	69.2	9.3	4.2	4.7	3.8	1.3
Hard white	66.7	14.7	19.9	11.5	17.4	23.0
Foam white	61.5	4.6	4.5	2.8	2.8	3.8
Soft blue/green	56.4	4.2	4.1	2.4	2.4	1.0
Hard transparent	48.7	8.3	9.3	4.3	9.0	10.9
Hard black/brown	46.2	5.1	9.2	3.3	2.7	5.7
Hard yellow	46.2	4.6	6.3	2.5	3.6	4.8
Hard red/pink/orange	46.2	4.0	6.6	2.4	3.1	4.3
Line transparent	46.2	4.6	0.8	1.2	1.7	0.3
Hard blue/green	38.5	12.6	15.8	5.5	5.3	7.8
Rubber red/pink/orange	38.5	3.2	7.0	2.0	0.9	1.6
Line white	30.8	3.6	5.1	1.3	1.3	1.6
Rope white	25.6	3.6	6.7	1.3	0.8	1.1

Soft colorful	25.6	2.1	1.1	0.4	0.6	0.2
Charcoal black/brown	23.1	4.0	5.6	1.1	0.6	0.8
Rubber black/brown	23.1	1.3	3.2	0.5	0.4	0.6
Line colorful	20.5	2.5	6.0	0.9	0.8	1.6
Line black/brown	20.5	1.4	1.3	0.3	0.4	0.4
Soft red/pink/orange	20.5	1.3	0.9	0.2	0.3	0.2
Foam yellow	17.9	7.6	9.2	1.5	1.2	1.8
Rubber yellow	17.9	1.1	2.3	0.3	0.3	0.6
Hard gray	15.4	4.2	5.7	0.8	1.6	3.3
Rubber blue/green	12.8	2.6	3.7	0.4	0.3	0.4
Soft yellow	12.8	2.2	2.1	0.3	0.3	0.3
Rope blue/green	10.3	3.2	8.0	0.6	0.2	0.3
Foam black/brown	7.7	1.7	0.7	0.1	0.1	0.1
Line yellow	7.7	2.0	0.1	0.1	0.2	<0.1
Line red/pink/orange	7.7	1.3	0.1	0.1	0.1	<0.1
Rope black/brown	5.1	3.4	8.7	0.3	0.1	0.4
Other white	5.1	3.8	2.8	0.2	0.1	0.1
Foam transparent	5.1	2.9	2.4	0.1	0.1	0.1
Soft gray	5.1	2.4	2.3	0.1	0.1	0.1
Other blue/green	2.6	1.7	3.7	0.1	<0.1	0.1
Rubber white	2.6	1.2	1.0	<0.1	<0.1	<0.1
Rubber transparent	2.6	1.1	1.1	<0.1	<0.1	<0.1
Rope gray	2.6	0.2	0.2	<0.1	<0.1	0.1

Table S2. Types and colors for marine litter ingested by loggerhead turtles *Caretta caretta*. FO% = frequency of occurrence, PN% = prey-specific numerical percentage, PM% = prey-specific mass percentage, PSIRI% = Prey-Specific Index of Relative Importance, %N = numerical percentage and %M = mass percentage.

Caretta caretta						
Type	**FO%**	**PN%**	**PM%**	**PSIRI%**	**%N**	**%M**
Soft	42.9	46.2	36.5	17.7	8.8	0.7
Hard	42.9	57.3	80.9	29.6	23.5	47.3
Foam	28.6	57.9	70.0	18.3	14.7	7.4
Rubber	14.3	100.0	100.0	14.3	8.8	40.8
Line	14.3	73.7	8.1	5.8	41.2	1.4
Paper	14.3	100.0	100.0	14.3	2.9	2.22
Color						
White	57.1	64.9	75.3	40.1	26.5	57.8
Black/Brown	42.9	70.2	67.2	29.4	17.6	2.9
Transparent	28.6	36.0	46.5	11.8	8.8	2.9
Blue/Green	14.3	57.9	4.2	4.4	32.4	0.7
Red/Pink/Orange	14.3	100.0	100.0	14.3	14.7	35.7