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Bridging the gap: reviewing classification of plastic debris ingested by sea turtles

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Essa dissertação está estruturada em três capítulos. O primeiro é uma introdução geral, apresentada em Português e Inglês. O segundo capítulo corresponde a parte principal da dissertação e está no formato de um artigo. O terceiro é uma conclusão geral, apresentada também em Português e Inglês

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CHAPTER I

General introduction

1. Apresentação

Detritos marinhos de origem antrópico podem ser encontrados desde o fundo a superfície marinha e em praias oceânicas ao redor do mundo (Suaria e Aliani, 2014). A maior parte das pesquisas se concentrou nos detritos flutuantes e muito pouco nos detritos de regiões profundas. Portanto era crença comum que a maioria do plástico se mantém na superfície. No entanto, hoje é estimado que 70% do detrito plástico é depositado no fundo oceânico (Pham et al., 2014). Muitos estudos recentes demonstram que áreas profundas do oceano contém uma grande quantidade de detrito plástico (Angiolillo et al., 2015; Pasquini et al., 2016; Pham et al., 2014; Ramirez-llodra et al., 2013; Watters et al., 2010). A flutuabilidade dos detritos depende da composição de polímeros e da presença de ar ou sedimentos retidos no detrito. (Engler, 2012) Bioincrustação e percolação aditiva também podem alterar a densidade do detrito (Andrady, 2015; Galloway et al., 2017) Polipropileno e polietileno compõem a maior parte do detrito plástico flutuante pois são amplamente produzidos e têm flutuabilidade, como mostrado na Tabela 2 (Galgani et al., 2015; Pham et al., 2014; Suaria and Aliani, 2014). Tipos de plástico com densidade mais alta que a água do mar somente flutuarão se eles tiverem ar retido.

A ingestão de detritos plásticos é um problema reconhecido e crescente, que gera efeitos deletérios em muitos animais marinhos (Gall and Thompson, 2015). A probabilidade de encontro e ingestão de plástico, bem como as consequências para a saúde da ingestão de plástico dependem da etapa do ciclo de vida da tartaruga (Nelms et al., 2016). O ciclo de vida de uma tartaruga-marinha inclui diferentes habitats: de praias onde a oviposição e o desenvolvimento embrionário ocorrem até zonas neríticas e oceânicas, onde recrutamento e forrageamento acontecem (Bolten, 2003). A ingestão de detrito plástico pode causar efeitos letais ou subletais em tartarugas-marinhas. É mais difícil detectar efeitos subletais, mesmo que sejam provavelmente mais frequentes (Clukey et al., 2018; Jerdy et al., 2017; Nelms et al., 2016; Schuyler et al., 2014). Geralmente, somente quando existe dano aparente ao sistema digestivo e obstrução é possível relacionar diretamente a morte de um indivíduo com a presença de plástico (Jerdy et al., 2017). Particularmente, fragmentos duros e linhas de pesca podem causar ferimentos internos e obstrução intestinal quando eles passam pelo trato gastrointestinal da tartaruga-marinha depois que são ingeridos (Nelms et al., 2016). No entanto, a frequência destes efeitos tem grandes variações. Por exemplo, Jerdy et al. (2017) analisaram 65 tartarugas-marinhas encalhadas no sudeste do Brasil e 11 delas tinham perfurações por um objeto afiado e 54 apresentaram lesões devido a impactação. Contudo, Clukey et al. (2017) não encontraram lesões, inflamações ou perfurações no trato gastrointestinal de 55 tartarugas provindas de pescarias de espinhel do Pacífico, mesmo que 91% delas tenha ingerido plástico. Este exemplo sugere que quantidades significativas de detritos podem se acumular e ficar retidos dentro do trato gastrointestinal sem causar danos letais (Katharine E. Clukey et al., 2017). A probabilidade de obstrução intestinal devido à ingestão de detrito plástico varia de acordo com a espécie: Casale et al. (2016) descobriram que a tartaruga-cabeçuda são menos vulneráveis a quantidades pequenas de plástico que outras espécies. Por outro lado, Santos et al. (2015) descobriram que somente 0.5g de detrito plástico pode causar a morte de tartarugas-verdes imaturas, como aconteceu com duas das 265 amostras coletadas na costa brasileira.

Contaminação química é outro impacto causado pela ingestão de detrito marinho. Plástico contém aditivos variados (e.g. retardantes de chamas, estabilizantes, pigmentos e preenchimentos), que podem percolar quando ingeridos e ser absorvidos nos tecidos (Keller et al., 2004; Nelms et al., 2016; Rochman, 2015). Particularmente, Bisfenol A e ftalatos podem agir como disruptores endócrinos (Oehlmann et al., 2009). Efeitos subletais como diluição alimentar podem causar uma redução no estímulo para alimentação e na capacidade estomacal, que eventualmente leva a subnutrição (Katharine E. Clukey et al., 2017). Isto pode causar a depressão do sistema imunológico e uma maior vulnerabilidade a doenças, como fibropapilomitose, que é debilitante e até fatal (Nelms et al., 2016). Ocorrências desta doença foram documentadas em tartarugas-marinhas que haviam ingeridos detritos antropogênicos (Foley et al., 2007; Santos et al., 2011).

Nas últimas duas décadas, a documentação da ingestão de plástico pelas tartarugas marinhas tem aumentado exponencialmente e espera-se que aumente à medida que a quantidade de plástico que entra no oceano continue a crescer (Nelms et al., 2016). No entanto, há pouca consistência nos métodos usados para classificar os detritos plásticos ingeridos, dificultando comparações espaciais e temporais nos estudos de tartarugas marinhas em todo o mundo (Provencher et al., 2017). A maioria dos métodos usados para classificar os detritos plásticos baseia-se unicamente na aparência física dos detritos, reduzindo as informações disponíveis sobre o tipo de detritos plásticos ingeridos pelas tartarugas marinhas. É importante também examinar características não morfológicas, como a densidade e a composição do polímero, porque essa informação aumenta nossa compreensão dos fatores que fazem com que as tartarugas marinhas comam detritos plásticos. O objetivo deste estudo é identificar lacunas nos métodos de classificação de resíduos plásticos ingeridos por meio de levantamento bibliográfico. Analisamos 81 artigos científicos que estudam a dieta das tartarugas marinhas de 1998 a 2017 e avaliamos os métodos de classificação mais comuns. Particularmente, nós examinamos se os autores mediram os parâmetros básicos (abundância, cor) e se eles categorizaram os detritos plásticos ingeridos com base na textura (macia / dura) ou no tipo morfológico (corda, espuma, saco plástico). Na literatura pesquisada, encontramos que 14% (n = 11) dos estudos revisados não mencionaram fragmentos de plástico. Além disso, do total de 81 estudos revisados, apenas 57 analisaram resíduos plásticos ingeridos e desses estudos 86% (n = 49) não utilizaram um protocolo padrão. Quatorze estudos quantificaram somente os resíduos plásticos (massa, volume, frequência de ocorrência), sem classificá-los mais detalhadamente. Dos estudos que categorizaram resíduos plásticos ingeridos, vários não declararam claramente em seus métodos quais eram os critérios usados para classificar os detritos plásticos ingeridos. Resumimos as classificações mais utilizadas e verificamos que a maioria classificou por textura plástica (n = 15) enquanto 12 classificaram pelo tipo morfológico e oito usaram ambos. Com base nos resultados desta revisão da literatura, criamos uma árvore para tomada de decisão para ajudar os pesquisadores que estudam a ingestão de detritos plásticos pelas tartarugas marinhas a escolher o método de classificação mais apropriado, de acordo com o objetivo principal de seu estudo. Finalmente, discutimos e recomendamos técnicas padronizadas para classificar os resíduos plásticos com base em características não morfológicas. O objetivo final é melhorar a classificação de detritos plásticos e nossa compreensão dos efeitos e causas que levam as tartarugas marinhas a ingerir detritos plásticos.

2. General introduction

Plastics are a diverse group of petroleum-based synthetic polymers that started being produced in the 20th century (Pham et al., 2014). There are two main categories of plastics: thermoplastics and thermosets. Most consumer products are thermoplastics, such as polyethylene terephthalate (Niaounakis, 2017). They are formed by melting the plastic row material and they can be recovered and melted multiple times (Mohr et al., 2006). Instead, thermoset plastics, such as polystyrene, undergo a chemical change when heated (Mohr et al., 2006). After they are heated and formed thermosets cannot be re-melted (Mohr et al., 2006). Consequently, the recycling rate of thermosets is particularly low (Gourmelon, 2015).

In order to help consumers distinguish the different types of thermoplastics that compose the most common consumer goods, The Society of the Plastics Industry established in 1988 the classification system showed in Table 1. However, not all polymer types are included in this classification: an example are the polymer blends that compose cigarette filters, which are the most common marine and beach litter (Galgani et al., 2015). A downfall of this classification is that when a plastic product is classified as code 7, it can be anything from polycarbonate, nylon, acrylonitrile butadiene styrene (ABS) to layered or multi-material mixed polymers (Niaounakis, 2017) .

Table 1. The seven main categories of plastics established by the Society of the Plastics Industry (SPI), created to help consumers and recyclers to identify the different types of plastics (PlasticsEurope, 2017).

Plastic code	Name	Abbreviation	Common uses	Recyclability
1	Polyethylene terephthalate	PET	Water bottles	Commonly recycled
2	High-density polyethylene	HDPE	Bottle lids	Commonly recycled
3	Polyvinyl chloride	PVC	Building material	Sometimes recycled
4	Low-density	LPDE	Plastic bags	Sometimes

	polyethylene			recycled
5	Polypropylene	PP	Reusable food containers	Occasionally recycled
6	Polystyrene	PS	Foam packaging	Commonly recycled
7	Other (e.g., polycarbonate, nylon 6)	PC, PA6	CDs and DVDs, ropes	Difficult to recycle

Plastics debris can be classified based on size. Several definitions of nanoplastic and microplastic have been suggested (da Costa et al., 2016). We chose to follow the definition of microplastic by Galgani et al. (2013), which says that microplastic is in the size range 1-5 mm, while we consider nanoplastic as any plastic debris in the size range from 1 to 1000 nm (Gigault et al., 2018). Currently microplastics and nanoplastics are the most common form of plastic debris in the ocean (Galloway et al., 2017; Ivar Do Sul and Costa, 2014). Nanoplastics are thought to be the result of degradation of larger plastic debris (Gigault et al., 2018). For microplastic, two categories exist: primary and secondary. The former include plastic products that were manufactured to be small, such as pellets and microbeads (Galloway et al., 2017). The latter is a result of fragmentation of larger pieces of plastic debris in the environment, via weathering mechanisms such as photooxidation, biodegradation and mechanical action (Moore, 2008).

1.1. Distribution of plastic debris in the ocean

Marine litter can be found on the bottom and surface of the ocean and in beaches all over the world (Suaria and Aliani, 2014). Most research used to focus on floating rather than deep-sea debris and thus it was widely assumed that most plastic stays on the ocean surface. However, it has been estimated that 70% of plastic debris sinks to the seafloor (Pham et al., 2014). Many recent studies have shown that deep-sea areas contain a large quantity of plastic debris (Angiolillo et al., 2015; Pasquini et al., 2016; Pham et al., 2014; Ramirez-llodra et al., 2013; Watters et al., 2010)

Debris buoyancy depends on the polymer composition and on the presence of entrapped air or sedimentation (Engler, 2012). Biofouling and additive leaching can also change debris density (Andrady, 2015; Galloway et al., 2017). Polypropylene and polyethylene compose most of the floating plastic debris since they are widely produced and positively buoyant, as shown in Table 2 (Galgani et al., 2015; Pham et al., 2014; Suaria and Aliani, 2014). Plastic types with higher density than seawater will only float if they have entrapped air.

Table 2. Plastics type densities compared to seawater density (Engler, 2012). Notice that only PP and LDPE have lower density than seawater.

Polymer type	**Density (g/ml)**
Seawater	1.02-1.03
Polypropylene (PP)	0.85-0.92
Polyethylene (LDPE)	0.92-0.96
Polystyrene (PS)	1.04-1.08
Polycarbonate	1.36
Polyvinyl chloride (PVC)	1.16-1.41
Polyethylene terephthalate (PET)	1.38-1.41

Another major factor that determines plastic debris buoyancy is biofouling, which increases the specific density of the item (Galloway et al., 2017; Kaiser et al., 2017). Biofouling is defined as the accumulation of organisms and organic matter on submerged material (Engler, 2012; Kaiser et al., 2017; Kooi et al., 2017; Morét-Ferguson et al., 2010). Floating plastic debris can sink when affected by biofouling. Kaiser et al. (2017) have shown that microscopic biofilm alone is not enough to cause sinking of plastic particles: attachment of fouling macro-organisms is necessary.

As seawater density gradually increases with depth, plastic debris does not necessarily sink directly, but instead can stay suspended at the depth where its density equals seawater density (Kooi et al., 2017). This phenomenon explains why plastic particles are found throughout the water column (Li et al., 2016). Additionally, the time it takes for a particle to sink is density and size dependent (Kooi et al., 2017). Larger particles sink faster, while extremely small particles (<10μm) may take decades before settling on the ocean floor, persisting in the water column (Kooi et al., 2016).

In general, most litter found at shallow coastal water comes from beaches, coastal human settlements, aquaculture installments and river mouths (Pasquini et al., 2016; Pasternak et al., 2017). On the contrary, marine debris found in deep-sea waters likely originates from fishing and transportation vessels (Watters et al., 2010). More specifically, it appears that high litter density hotspots are associated with the most used shipping and fishing routes (Pasquini et al., 2016). However, plastic debris can also be found far away from waste sources. Gyres in particular can become plastic accumulation zones (Eriksen et al., 2013). According to Cozar et al. (2014) floating microplastics are present in all subtropical gyres. They estimated that there are tens of thousands of tons (10,000-40,000) of plastic in the surface layer of the open ocean

(Cozar et al., 2014) and the gyre with the biggest amount of plastic debris is currently in the South Pacific (Eriksen et al., 2013).

1.2. Plastic ingestion by sea turtles

For several decades, it has been known that marine debris, plastics in particular, affect marine organisms (Gall and Thompson, 2015; Kühn et al., 2015). One of the most affected taxa are sea turtles: all seven species were found to have some impact of plastic debris, either by entanglement or ingestion (Nelms et al., 2016). The ingestion of plastic debris can cause lethal or sub-lethal effects to sea turtles. Generally, it is more challenging to detect sub-lethal effects, even though they are likely to be the most frequent (Clukey et al., 2018; Jerdy et al., 2017; Nelms et al., 2016).

Only when there is apparent damage to the digestive system and obstruction it is possible to directly link the sea turtle death to the presence of plastic (Jerdy et al., 2017). In particular, hard fragments and fishing lines can cause internal injuries and intestinal blockage when they pass through the sea turtle gut after they have been ingested (Nelms et al., 2016). However, the frequency of these effects varies greatly. For example, Jerdy et al. (2017) analyzed 65 sea turtles stranded in southeastern Brazil and 11 of them had perforations by a sharp object and 54 presented lesions due to impaction. On the contrary, Clukey et al. (2017) found no lesions, inflammations or perforations in the gastrointestinal tract of 55 turtles from Pacific long line fisheries they analyzed, even though 90.9% of them had ingested plastic. This example shows that significant quantities of plastic debris can accumulate and remain within the gut without causing lethal damages (Clukey et al., 2017). Moreover, the probability of intestinal blockage due to plastic debris ingestion varies per species: Casale et al. (2016) have found that loggerheads are less vulnerable to small quantities of plastic than other species. Contrarily, Santos et al. (2015) have found that just 0.5 g of plastic debris can cause death in juvenile green turtles, as it happened to two of the 265 samples they collected along the Brazilian coast.

Chemical contamination is another impact caused by the ingestion of marine debris (Kühn et al., 2015). Plastics contain several additives (e.g. flame retardants, stabilizers, pigments and fillers), which can leach out when ingested and can be absorbed into tissues (Keller et al., 2004; Nelms et al., 2016; Rochman, 2015). In particular, Bisphenol A and phthalates can act as endocrine disrupters (Oehlmann et al., 2009). Moreover, sub-lethal effects such as dietary dilution can cause a reduction in feeding stimulus and stomach capacity, which eventually leads to malnutrition (Clukey et al., 2017). This, in turn, may cause a depressed immune system and an increased vulnerability to diseases, such as fibropapillomatosis, which is debilitating and often fatal (Nelms et al., 2016). Occurrence of this disease has been documented in sea turtles that had ingested anthropogenic debris (Foley et al., 2007; Santos et al., 2011).

The probability of plastic encounter and the health consequences of plastic ingestion depend on the turtle's life stage (Nelms et al., 2016). A sea turtle life cycle encompasses different habitats: from beaches where oviposition and embryonic development occur to neritic and oceanic zones, where recruiting and foraging happens (Bolten, 2003).

Little is known about the ecology and location of sea turtles at early juvenile stages and this is why it is also called the "lost year" (Bolten, 2003). However, we do know that most post-hatchling turtles, with the exception of the flatback turtle (*Natator depressus*) are pelagic and oceanic. Boyle & Limpus (2008) found that in the South-West Pacific neonate green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles have a similar diet based on oceanic mollusks and crustaceans, which reflects an opportunistic pelagic feeding ecology. Once in the oceanic environment, post-hatchling turtles are thought to follow converging border currents and regions of upwelling with high productivity (Arthur et al., 2008; Boyle and Limpus, 2008; Moore, 2008; Nelms et al., 2016). These are the same oceanic zones where most plastic debris accumulates (Cozar et al., 2014; Eriksen et al., 2013; Nelms et al., 2016). Consequently, the generalist diet and feeding area make neonate turtles particularly susceptible to plastic ingestion. Moreover, the low size and high fragility of their digestive tract may increase the risk of mortality from plastic ingestion (Ryan et al., 2016; Schuyler et al., 2014).

As sea turtles grow, their diets gradually become more specialized. Green turtles are primarily herbivorous (Scherer et al., 2014), while loggerheads and Kemp's ridley (*Lepidochelys kempii*) are carnivorous, mainly eating hard-bodied organisms such as crustaceans and mollusks (Bolten, 2003). Flatback turtles are also carnivorous but they primarily feed on soft-bodied organisms (Bolten, 2003). Olive ridley (*Lepidochelys olivacea*) and hawksbill turtles (*Eretmochelys imbricata)* are omnivorous but research has shown that hawksbills mainly feed on sponges (Meylan, 1988). Leatherbacks (*Dermochelys coriacea*), instead, feed exclusively on jellyfish (Bolten, 2003).

Usually a change of diet is associated with a change in life history stage. Each species has its own life history pattern, which is distinguished in developmental (oceanic vs. neritic) and adult foraging (oceanic vs. neritic) stage (Bolten, 2003). The flatback turtle is the only species that spends its whole life in the same habitat: the neritic zone (Bolten, 2003). At the post-hatchling stage, flatback turtles probably mostly live close to the ocean surface and as they grow they might develop a benthic foraging strategy (Bolten, 2003). On the contrary, leatherbacks spend both their developmental and adult stage in the oceanic zone (Bolten, 2003).

Loggerheads spend their early developmental stage in the oceanic zone and later switch to the neritic zone (Musick and Limpus, 1997). In particular, juvenile loggerheads turtles seem to prefer convergence zones and major gyre systems (Musick and Limpus, 1997). Other four sea turtle species have the same life cycle pattern as loggerheads: hawksbill, green turtle, Kemp's ridley and olive ridley (Bolten, 2003). However, this life cycle pattern is not always the rule. There have been reports of female olive ridley turtles staying in the oceanic zone after nesting, instead of going back to the neritic zone (Godley et al., 2008). There have also been cases of loggerhead adults staying in the oceanic zone instead of moving to the neritic zone for the adult foraging stage (Polovina et al., 2004).

Overall, it is clear that all seven species of sea turtles have high probability of encountering marine debris because most of them inhabit both the neritic and oceanic zone at some point in their lives. Moreover, most of the species are migratory and this also increases their probability of marine debris encounter.

1.3. Objectives and summary of the results

In the last two decades documentation of plastic ingestion by sea turtles has been increasing exponentially and it is expected to rise as the quantity of plastic entering the ocean continues to grow (Nelms et al., 2016). However, there is little consistency in the methods used to classify ingested plastic debris, hindering spatial and temporal comparisons of sea turtle studies across the world (Provencher et al., 2017). Most of the methods used for classifying plastic debris are based solely on the physical appearance of debris, reducing the available information on the type of plastic debris ingested by sea turtles. It is important to also examine non-morphological characteristics, such as density and polymer composition because this information increases our understanding of the factors that cause sea turtles to eat plastic debris. The objective of this study is to identify gaps in classification methods of ingested plastic debris through a literature survey. We analyzed 81 scientific articles studying sea turtle diet from 1998 to 2017 and assessed the most common classification methods. Particularly, we examined whether the authors measured basic parameters (abundance, color) and whether they categorized ingested plastic debris based on either texture (soft/hard) or morphological type (rope, foam, plastic bag). In the surveyed literature we found that 14% (n=11) of the reviewed studies did not mention plastic debris. Moreover, out of the total 81 reviewed studies, only 57 analyzed ingested plastic debris and of these studies 86% (n=49) did not use a standard protocol. Fourteen studies solely quantified ingested plastic debris (mass, volume, frequency of occurrence), without classifying it further. Of the studies that did categorize ingested plastic debris, several did not state clearly in their methods which were the criteria they used to classify ingested plastic debris. We summarized the most used classifications and found that most studies classified by plastic texture (n=15) while 12 classified by morphological type and eight used both. Based on the results of this literature review, we created a decision-making tree for helping researchers studying plastic debris ingestion by sea turtles to choose the most appropriate classification method according to the main objective of their study. Finally, we discussed and recommended standardized techniques to classify ingested plastic debris based on non-morphological characteristics. The ultimate aim is to improve ingested plastic debris classification and our understanding of the effects and causes that lead sea turtles to ingest plastic debris.

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CHAPTER II

Bridging the gap: reviewing classification of plastic debris ingested by sea turtles

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Bridging the gap: reviewing classification of plastic debris ingested by sea turtles

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Abstract

Plastic pollution of oceans is a global issue. Sea turtle debris ingestion has been widely documented, but there is little consistency in the methods used to classify ingested plastic debris and most solely measure plastic debris morphology. Examining physical characteristics of plastic debris will increase our ability to identify the factors that affect sea turtle vulnerability to plastic pollution. We analyzed 81 scientific articles studying sea turtle diet from 1998 to 2017 and assessed the most common classification methods. Only 57 papers analyzed ingested plastic debris and of these studies 49 did not use a standard protocol. Most publications that classified ingested plastic debris examined the texture (43%), while 34% classified by morphological type and 23% used both. Based on the results of this literature review, we created a decision-making tree for choosing the method most fit to the study purpose. Finally, we recommend new approaches to study sea turtle plastic ingestion.

Keywords: ingestion of marine debris, marine turtles, standard protocol, microplastic.

Resumo

A poluição plástica dos oceanos é um problema global. A ingestão de detritos por tartarugas-marinhas é amplamente documentada, mas existe pouca consistência nos métodos utilizados para classificar detritos plásticos ingeridos e a maioria somente descreve a morfologia dos detritos. Examinar características físicas dos detritos plásticos aumentará nossa habilidade de identificar os fatores que afetam a vulnerabilidade das tartarugas-marinhas à poluição dos oceanos por plásticos. Nós analisamos 81 artigos científico estudando dietas de tartarugas-marinhas de 1998 a 2017 e avaliamos os métodos de classificação mais comuns. Somente 57 artigos analisaram os detritos plásticos ingeridos e destes estudos 86% não utilizaram um protocolo padrão. A maioria das publicações quantificam os detritos plásticos de acordo com a textura (43%), enquanto 34% classificam por tipo morfológico e apenas 23% pelos dois. Baseado nos resultados desta síntese bibliográfica, criamos uma árvore de decisões para escolher o método que melhor se aplica ao objetivo do estudo. Finalmente, recomendamos novas abordagens ao estudo da ingestão de plástico por tartarugas-marinhas.

Palavras-chaves: ingestão de detritos marinos, tartarugas marinhas, ingestão, protocolo padrão, microplástico.

1. Introduction

Plastics are a diverse group of petroleum-based synthetic polymers that started being produced in the 20th century (Pham et al., 2014). In the last 60 years, annual global plastic production has been increasing exponentially, reaching 335 millions of tons in 2016 (PlasticsEurope, 2017). Due to plastics relatively low price and convenience, the demand for this durable and multi-use material is rising (Gourmelon, 2015). The ubiquity of this material, added to the mismanagement of its disposal, has resulted in an exponential increase of plastic entering the ocean (Andrady, 2015). Currently, eight million tons of plastics are flowing into the sea every year (Jambeck et al., 2015). Most debris found in the sea has land origin and is transported to the sea by waterways, sewage, drainage systems and wind (Andrady, 2015; Jambeck et al., 2015). Marine debris is a wide group of different materials that include non-plastic products, such as tar, glass and metal. However, plastic is the most common type of marine debris, both in terms of ocean distribution and marine biota interaction (Ryan, 2015; Sigler, 2014). Therefore, in this literature review we decided to focus on plastic debris.

Plastic litter heavily affects marine organisms (Kühn et al., 2015). Specifically, 54% of all marine mammals species, 56% of all seabird species and all species of sea turtles, have either eaten or become entangled in marine debris (Gall and Thompson, 2015). All sea turtle species are of conservation concern and have become a focus for mitigation and awareness efforts of plastic pollution (Eagle et al., 2016), which explains why in the last couple of decades there has been particular focus on plastic debris ingestion by sea turtles (Galgani et al., 2013; Nelms et al., 2016; Pham et al., 2017; Schuyler et al., 2014a; Vélez-Rubio et al., 2017).

Since 2011 there have been attempts to harmonize methods in this research area (Galgani et al., 2013; MSFD GES Technical Subgroup on Marine Litter, 2011; van Franeker et al., 2011). It is particularly important to follow a standardized protocol due to the global range that nearly all sea turtle species have (Schuyler et al., 2014a). However, there is still little consistency when classifying plastic debris. Several studies have discussed the importance to standardize measurements to classify plastic debris ingested by sea turtles (Casale et al., 2016; Fossi et al., 2018; Nelms et al., 2016;

Provencher et al., 2017). In particular, Provencher et al. (2017) have given systematic metric recommendations for reporting ingested plastic debris in marine megafauna, with marine birds as a case study. However, a review that specifically focuses on how to improve methods to report plastic debris ingestion in sea turtles is lacking. Not only these methods require standardization, they also require measuring physical characteristics of plastic debris, such as chemical composition and density, rather than solely examining the morphology. This will increase our ability to fully understand the factors that cause sea turtles to ingest plastic and the context of sea turtle-plastic debris encounters under natural conditions.

The objectives of this study are to (i) identify the gaps in classification methods of ingested plastic debris in sea turtle research, (ii) recommend the use of methods that reflect the purpose of the study, including protocols that analyze the chemical and physical composition of plastic debris and (iii) discuss new approaches to close the methodological gaps found in the review and to increase our understanding of the causes that lead sea turtles to ingest plastic debris.

2. Methodology

2.1. Literature review

We systematically searched for literature regarding sea turtle diet and contamination associated with plastic debris. We examined the literature using Google Scholar search engine with citation index between 1998 and 2017 using the following keywords: *sea* or *marine turtle* followed by either *marine debris* and *plastic* or *diet* and *foraging ecology*. Studies on sea turtles' diet that did not consider plastic debris in their analysis were also included in this review. Studies that considered plastic debris but did not find any in the sampled sea turtles were also included in the literature review. We only reviewed studies in English. Furthermore, here microplastics and nanoplastics are considered to be within the broader category of plastics and thus are also included in this review. We chose to follow the definition by Galgani et al. (2013) for microplastic, which says that microplastic is in the size range 1-5 mm, while for nanoplastic we followed the definition by Gigault et al. (2018), which considers nanoplastic as any plastic debris in the size range from 1 to 1000 nm. Moreover, in our literature we included studies that analyze all marine debris, even though this means that also non-plastic debris are considered; we did not find this to be an issue because the vast majority of marine debris are plastic.

2.2. Data analysis

For each relevant publication, information was recorded regarding the main topic (research question, location, sample size, sampled species, whether the article focused on marine debris ingestion or on general sea turtle diet), whether the sampled turtles were found stranded, alive or as bycatch and the methods used to extract data on sea turtle diet (necropsy, feces, lavage or keratin samples). If a necropsy was performed, we noted which part of the gastrointestinal tract was examined (esophagus, stomach, intestine or total). Moreover, the methods employed to classify ingested plastic debris were recorded for each publication; in particular, we noted which characteristics were examined: color, texture (hard/soft), mass, surface area, length, volume, density, polymer composition, number of pieces; furthermore, we recorded whether microplastics (when it was not specified we assumed microplastics were analyzed when the mesh size was smaller than 5 mm) and frequency of occurrence (%FO) were included in the study. Additionally, we documented whether the authors of each publication used a standardized protocol or if they classified plastic debris differently.

We recorded the main discussion topics for each reviewed publication. In particular, we noted whether the authors considered the fact that sea turtles eat plastic debris because it resembles jellyfish as a valid explanation for plastic debris ingestion (Schuyler et al., 2014b) or instead if they believed that white/transparent sheet plastic was more widely found in the ocean than other types of plastics and thus it was eaten more frequently by sea turtles. Moreover, we examined whether the authors of each relevant publication thought that sea turtles ingested plastic debris because it was mixed with their natural food. We also recorded whether, at any point in the discussion, the authors mentioned at which level of the water column sea turtles might have ingested plastic litter (benthic or pelagic). Furthermore, we noted whether they mentioned the effects that plastic debris ingestion has on sea turtle health. Finally, we used the data we gathered from the review of the publications' discussions to create a decision-making tree for methods of plastic debris classification.

3. Results and discussion

3.1. Description of the reviewed literature

Eighty-one publications were found in total that met our criteria (Supplementary material). Overall, the number of reviewed papers increased from 2011 onwards, when eight studies on sea turtle diet were found. The year with the highest number of publications (n=12) was 2016, of which six focused on plastic debris ingestion. Papers with study of plastic debris ingestion by sea turtles as a main objective started increasing from 2014 onwards (Fig.1). This growth reflects a general surge in interest in sea turtle plastic debris ingestion (Nelms et al., 2016). Nevertheless, studies that analyzed sea turtle diet but did not mention plastic debris (including negative results) were found as recently as 2016 (Fig.1).

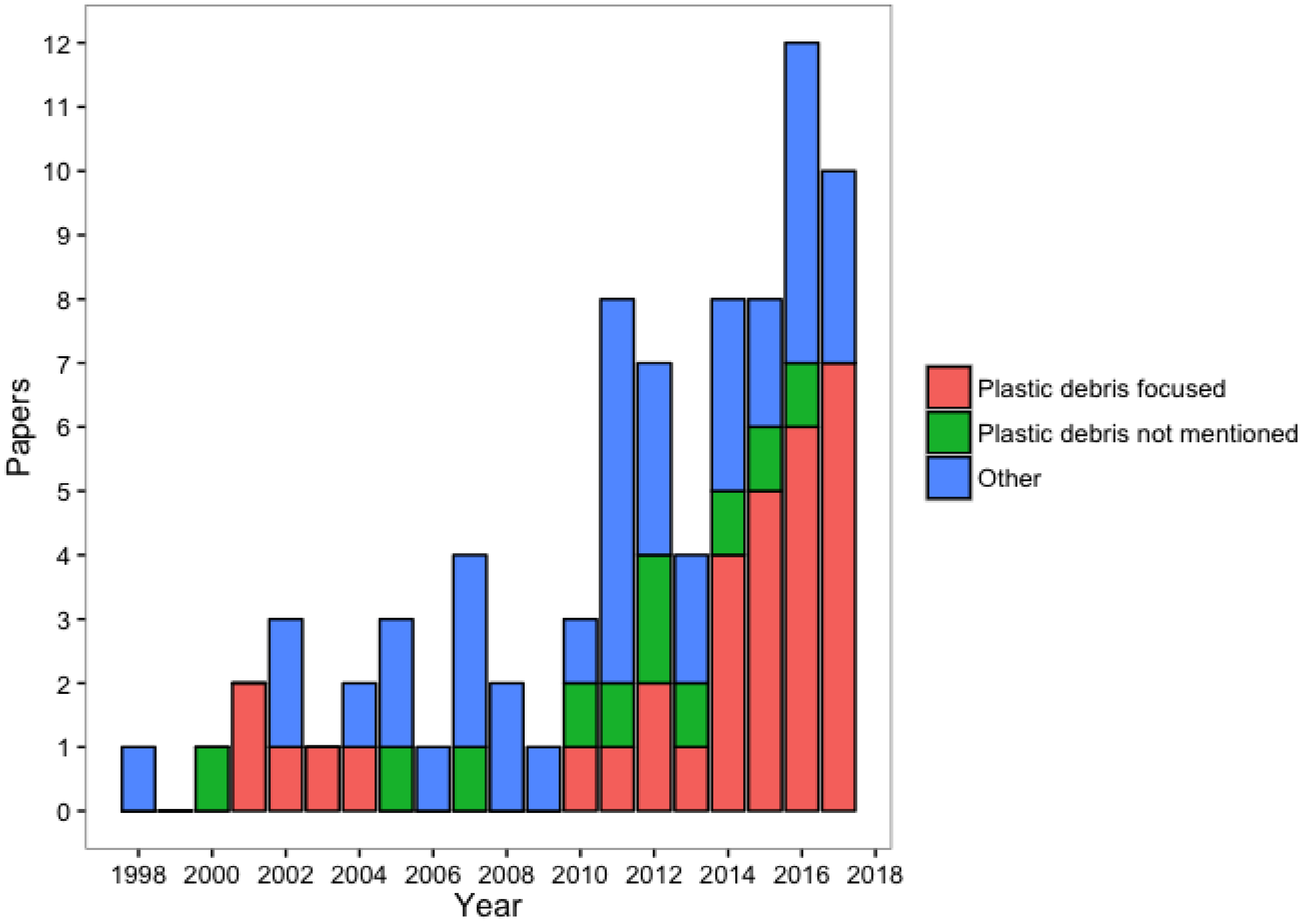

Figure 1. Total reviewed papers from 1998 to 2017 (n=81), classified in: papers focused on plastic debris ingestion by sea turtles (n=32), papers on sea turtle diet analysis not mentioning plastic debris (n=11) and papers not focused on plastic debris ingestion by sea turtles but mentioning it in their diet analysis (other, n=38).

3.2. Biases in studies on sea turtle plastic ingestion

In the 81 publications that we reviewed we noticed three main biases: (i) Taxonomic - loggerhead and green turtles are the most studied species, (ii) Geographic - there are very few studies in the Indian Ocean especially when comparing to the Atlantic Ocean (as shown in Figure 2) and (iii) Methodological - a partial necropsy of the gastrointestinal tract can lead to an underestimation of the occurrence of ingested plastic debris.

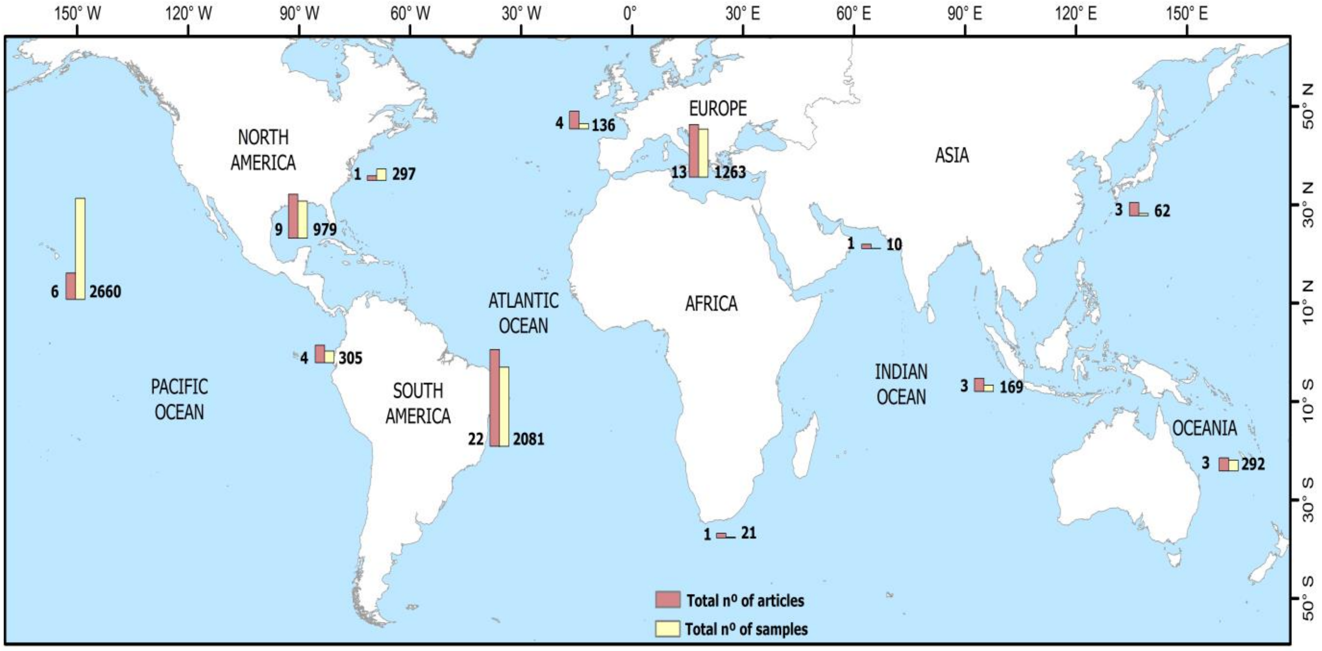

Figure 2. Location of studies that mention sea turtle plastic debris ingestion, including the ones that did not find any in their sample (n=70). Pink columns indicate the total number of articles and yellow columns indicate the total number of samples. The columns are proportionate inside the categories (for geographic comparison), but not among categories (articles x samples). All sea turtles species are included.

Most of the reviewed literature that considered plastic debris ingestion (n=70) focused on green turtles (n=45) and loggerheads (n=28). The remaining five species are still understudied. Only eight of the 70 articles that consider plastic debris sampled leatherbacks. Kemp’s ridley, hawksbill and olive ridley are even more understudied, with only four, five and six studies respectively. For flatbacks, there is only one article (Schuyler et al., 2012). This could be explained by their restricted geographic distribution. It is worth noticing that we observed the same taxonomic bias reported by

Nelms et al. (2016), showing that this bias has persisted in more recent publications (from 2015 to 2017), which represent more than a third of the total literature we reviewed (n=28). Clearly more studies on these neglected species are needed, especially given the conservation status of flatbacks, hawksbills and Kemp's ridleys (Schuyler et al., 2014a).

Regarding geographical coverage, most studies that report plastic ingestion by sea turtles are concentrated in the Southern Atlantic Ocean and in the Mediterranean Sea (Fig. 2). More research is needed in the South Pacific and in the Indian Ocean. Moreover, it is not only the number of studies that matters but also the sample size. For example, the Northern Pacific Ocean has less than half of the studies of the Mediterranean but the total number of sampled sea turtles is more than double the Mediterranean. This is mainly due to one study in Hawaii with a particularly large sample (Russell et al., 2011). Nelms et al. (2016) observed a similar geographical bias in their literature review that spanned from 1985 to 2014. From 2015 to 2017 there has been some research on sea turtle plastic ingestion in under-studied areas, such as: South-Africa (Ryan et al., 2016), Japan (Fukuoka et al., 2016), Hong Kong (Ng et al., 2016) and the west coast of Australia (Reinhold, 2015); however, this bias remains and further research is still needed.

Of the 81 papers considered in this review, 66 of these included necropsy studies, but only 61% (n=40) analyzed the whole gastrointestinal tract (GI). The rest only examined some parts, generally the stomach or the intestine. This could significantly change the results of a study. For example, the abundance of plastic debris and lesions to the GI caused by the ingestion of plastic debris could go unreported. This could happen especially when only the stomach is analyzed, as often plastic debris accumulates more in the intestine than in the stomach (Casale et al., 2008; Tourinho et al., 2010).

3.3. Gaps in the methods for classifying ingested plastic debris

Papers included in our literature review analyzed ingested plastic debris (IPD) in many different ways. Often, though, the criteria used to classify ingested debris were not stated (e.g., de Carvalho et al., 2015; Poppi et al., 2012; Wedemeyer-Strombel et al., 2015) Moreover, many studies wrote that they classified IPD by "type" without explaining further the classification criteria (e.g., Gama et al., 2016; Guebert-Bartholo et al., 2011; Lazar and Gračan, 2011). To show the existing gaps in the methods for classifying IPD, we ordered them into categories and then organized the literature accordingly (Fig. 3).

Around 70% (n=57) of the publications that considered IPD analyzed IPD beyond the frequency of occurrence (%FO). However, it is important to notice that only 14% of these studies used the standard protocol created by Van Franeker et al. (2011) and recommended by the European Directory (Galgani et al., 2013; MSFD GES Technical Subgroup on Marine Litter, 2011). The remaining 49 studies analyzed IPD using other methods. In particular, 29% of these 49 studies only measured basic parameters of IPD. This means that they did not categorize plastic but they did examine its abundance by calculating total mass and/or volume or by counting the number of pieces. Here we also considered length, color and surface area of each plastic item as basic parameters. Thus if a study examined any of the basic parameters that were previously mentioned without classifying IPD in further categories, it was allocated in the category "only measure basic parameters of IPD" shown in Figure 3.

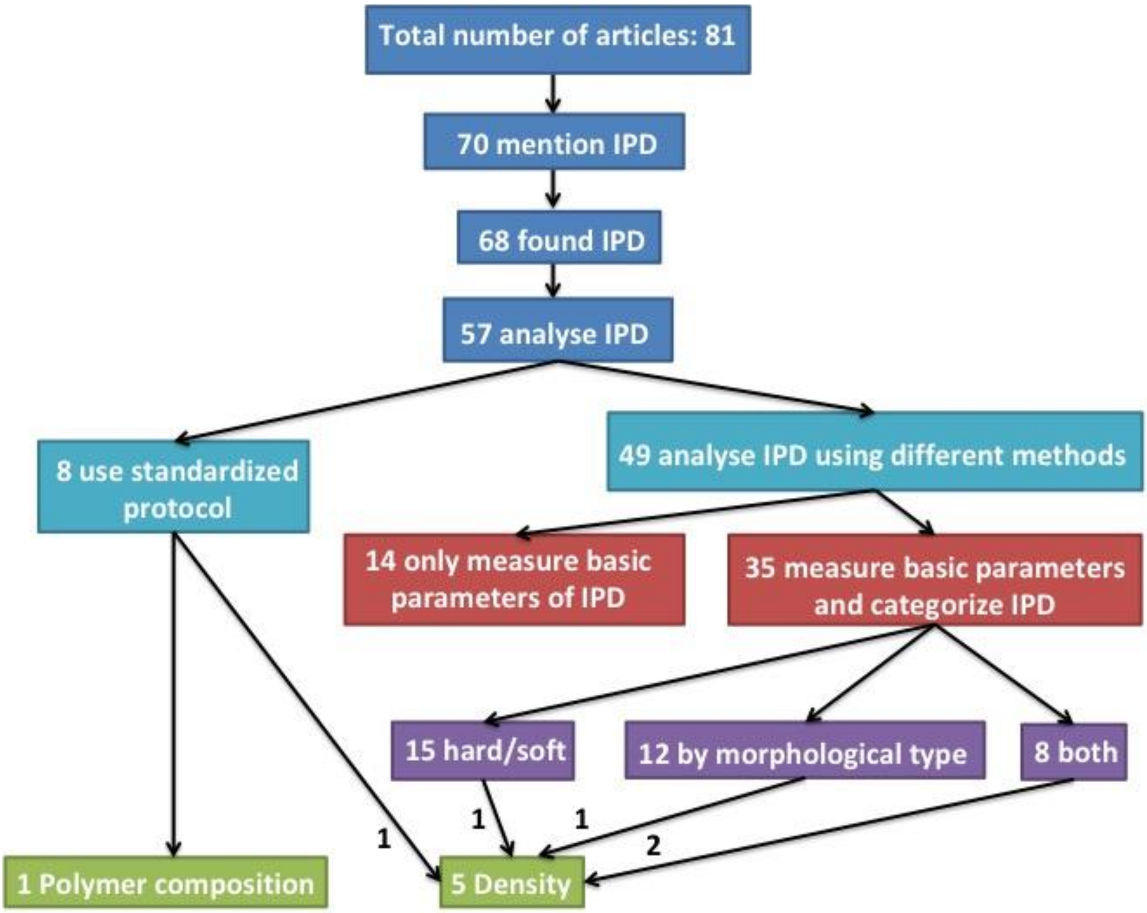

Figure 3. Categorization of the methodological approach implemented in the reviewed literature (1998-2017) regarding ingested plastic debris (IPD) by sea turtles.

Of the 49 studies that analyzed IPD using a different method than the one suggested by van Franeker et al. (2011), 71% categorized IPD besides measuring basic parameters. In the reviewed literature we found two main techniques to categorize IPD. The first one is based on plastic texture, and it separates plastic into ‘hard’ and ‘soft’. The second technique classifies IPD based on its morphological type; for example, if an item is thread-like then it will be classified as ‘rope’ or if it is sheet-like, it will be called ‘plastic bag’ (e.g., Casale et al., 2008; Ferreira et al., 2006). However, this classification varied amply in the literature. Moreover, 43% of the 35 papers that measured basic parameters and categorized IPD used the ‘hard’ /‘soft’ classification, while 34%

categorized IPD by morphological type. Finally, 22% mixed the two methods, for example they classified some items as ‘hard’ and others as ‘plastic bag’ pieces. It must be reminded, however, that some studies which used the ‘hard’/ ‘soft’ classification method excluded some plastic items from this type of categorization, such as fishing items (e.g., de Carvalho et al., 2015; Fukuoka et al., 2016; Lazar & Gračan, 2011). Furthermore, only six publications of the 57 that analyzed IPD measured non-morphological characteristics of IPD such as density and polymer composition.

From Figure 4 we can see that even though van Franeker et al. (2011) created a standardized protocol to classify ingested plastic debris in 2011, only in 2013 it was first used in sea turtle research. This could be explained by the fact that the methodology was originally developed for the analysis of plastic litter ingested by sea birds (in particular northern fulmars). Although the MSFD GES Technical Subgroup on Marine Litter already recommended the protocol for sea turtle research in 2011, our results suggest that only when Galgani et al. (2013) endorsed the protocol it began to be used in sea turtle research (Fig. 4). In 2015 none of the six reviewed publications that considered IPD used the standardized protocol, but its usage increased in 2016 and 2017. This protocol is implemented by European as well as non-European studies (Colferai et al., 2017; Vélez-Rubio et al., 2017).

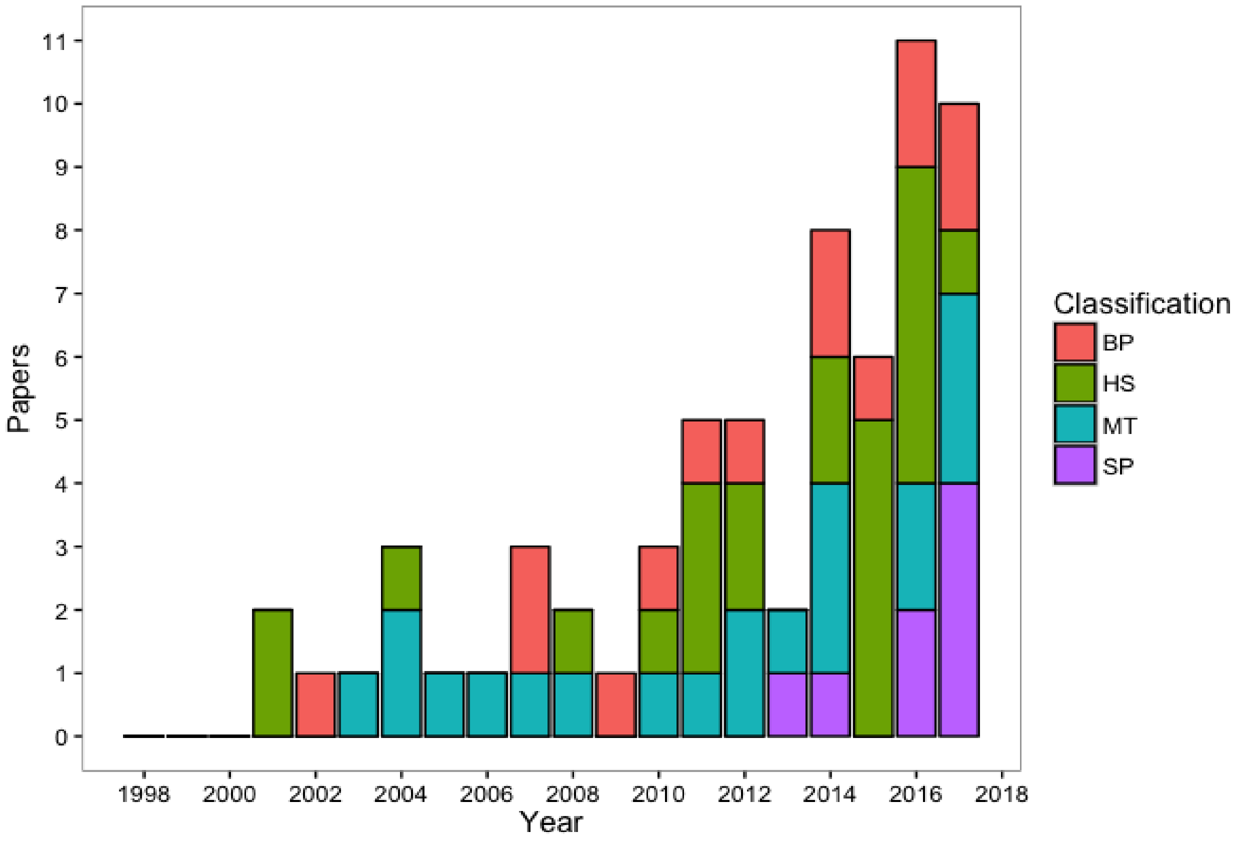

Figure 4. Use of classification methods of ingested plastic debris (IPD) by sea turtles in the literature from 1998 to 2017. Standardized protocol (SP), morphological type (MT), hard/soft texture (HS) and only basic parameters of IPD (BP). Papers that used both MT and HS were included in each category and thus counted twice overall.

3.3.1. Terminology used to describe ingested plastic debris

Across the 81 publications we reviewed, we noticed that there was a large inconsistency in the vocabulary used to describe ingested plastic debris. This tended to happen especially in studies that categorized IPD without following a standardized protocol. In particular, studies that classified IPD by morphological type used a wide range of names to describe plastic items. The most common morphological types were: foam, sheet-like plastic and thread-like plastic. However, in some studies foam and thread-like items were separated from the category of plastic debris and they were called 'fishing gear' (e.g., Fukuoka et al., 2016; Hoarau et al., 2014; Jerdy et al., 2017).

3.3.1.1. Plastic foam

Plastic foam is a common anthropogenic debris found in the ocean and in turtles' stomachs (Galgani et al., 2013; Schuyler et al., 2012; Suaria and Aliani, 2014). Plastic foam is a general term that includes many types of foams with various polymer compositions (Okoroafor and Frisch, 1995). They can have different physical characteristics: they may be flexible or rigid, they may have open or closed cell and have different densities (Okoroafor and Frisch, 1995). Here we assume that when studies use the term 'foam' they are referring to expanded polystyrene (EPS), which is less dense than water and it is one of the most used types of plastic (Andrady, 2015). Some studies call plastic foam 'polystyrene' (e.g., Guebert-Bartholo et al., 2011; Parker et al., 2005; Tomás et al., 2002; Tourinho et al., 2010) but we advise to only do so when the polymer composition of the plastic item is identified.

Some studies use the name 'Styrofoam' to refer to plastic foam (e.g., Fukuoka et al., 2016; Hoarau et al., 2014; Parker et al., 2005), which we assume it refers to expanded polystyrene. Styrofoam is a trademarked brand of closed cells extruded polystyrene foam (XPS) and therefore is a different foam type from expanded polystyrene (Okoroafor and Frisch, 1995). However, in the United States and Canada the word 'Styrofoam' has become a term for expanded polystyrene in colloquial language. Even though it is a Northern American colloquial use, the word 'Styrofoam' is used in sea turtle studies across the world. In our opinion, though, it is not a suitable word to refer to plastic foam because it is a name of a trademarked brand and not a technical word. The polymer composition of plastic foam should be identified through spectrometry or analysis of physical properties (buoyancy, flame colour) so that the correct terminology is used for the respective type of plastic foam. If the polymer composition cannot be assessed then we recommend naming the item 'foam-like' and describing in detail the morphological characteristics of the debris.

3.3.1.2. Rope

Another widely used morphological category is 'rope'. Several words are used besides 'rope': fishing line, monofilament, nylon and polypropylene line (e.g., Boyle & Limpus, 2008; Parker et al., 2011; Stahelin et al., 2012). Often 'rope' and 'nylon' are considered two different items, even though the difference between the two is often not explained (e.g., de Carvalho et al., 2015; Guebert-Bartholo et al., 2011; Revelles et al., 2007).

Nylon is a generic designation for a family of synthetic polymers joined together through condensation polymerization and it is one of the most common artificially made polyamides (Vagholkar, 2016). In common usage, the abbreviation 'PA' is used interchangeably with the word 'nylon'. When nylon is produced, its density is higher than seawater and thus it is expected to sink once it enters the ocean as debris (Engler, 2012). Some studies on plastic debris ingestion by sea turtles classify certain types of rope as 'polypropylene line' (e.g., Parker et al., 2011). Polypropylene is a different polymer than nylon (resin type 5) and its density is lower than seawater (Engler, 2012). In addition, equally to nylon, one of its many uses is for fishing equipment such as lines and nets (MSFD GES Technical Subgroup on Marine Litter, 2011).

Fishing lines and nets are some of the most common plastic debris found in the ocean (Galgani et al., 2015). Usually fishing lines are made of Nylon 610 and 612 (Vagholkar, 2016) and are generally monofilament, which means they are made from a single fibre of plastic. Some studies use solely the word 'monofilament' to refer to fishing lines, but this name refers to the structure, not to the material. In our opinion, a more suitable name for any thread-like debris is one that refers to its material (nylon, polypropylene, etc.). However, to do so the polymer composition of the line should be identified. If this is not possible then the item should be named 'thread-like' and its morphological characteristics should be described in detail.

In general, each study should be consistent with the vocabulary they use to describe plastic debris. That is, polymer names (nylon, polypropylene, polystyrene) should only be used when the chemical composition of debris is tested. Most of all, studies should be coherent with the terminology they use for morphological types. Some studies (e.g., Casale et al., 2008; Guebert-Bartholo et al., 2011; Hoarau et al., 2014; Tourinho et al., 2010) mix plastic debris categorization by their original product (fishing line, packaging sheet or plastic bag) with their physical appearance (rope, monofilament, flexible sheet, foam). This could create confusion in plastic debris classification and hamper comparisons between studies.

3.3.2. Microplastic

Plastics debris can be classified according to size. Several definitions of nanoplastic and microplastic have been suggested (da Costa et al., 2016). Currently microplastics and nanoplastics are the most common form of plastic debris in the ocean (Galgani et al., 2015; Ivar Do Sul and Costa, 2014). Nanoplastics are generally considered to be the result of degradation of larger plastic debris (Gigault et al., 2018). On the other hand, two categories of microplastic exist: primary and secondary. The former include plastic products that were manufactured to be small, such as pellets, microbeads, etc (Galloway et al., 2017). The latter is a result of fragmentation of larger pieces of plastic debris in the environment, via weathering mechanisms such as photooxidation, biodegradation and mechanical action (Moore, 2008). Plastic resin pellets are a common primary microplastic typically used to manufacture larger plastic items (Moore, 2008). Due to their ubiquitous use and the surge of plastic production, these were some of the earliest plastic pollutants and tellingly appear in the earliest records of marine plastic pollution (Carpenter et al., 1972; Kartar et al., 1973; Morris and Hamilton, 1974). However, the term microplastic was still not used at this time.

Focus on the pollution of secondary microplastics (marine litter derived from the fragmentation of larger plastic debris), only started in the early 2000's. In particular, the study by Thompson et al. (2004) was largely responsible for the recent resurgence of interest in the marine litter problem (Ryan, 2015). Thompson et al. (2004) showed that small plastic fragments and fibres are common in sedimentary habitats and they suggested that they are a product of the breakdown of larger items. Moreover, they called these plastic particles microplastics, which was one of the first times the word was used (Thompson et al., 2004).

The first publication in our literature review that included microplastics was in 2008 (Fig. 5), four years after the surge of microplastic research. However, it must be highlighted that most studies did not explicitly specify whether they included microplastics in their classification of plastic debris, but we were able to deduce this information in case they specified the mesh size they utilized to sieve the gut content they sampled.

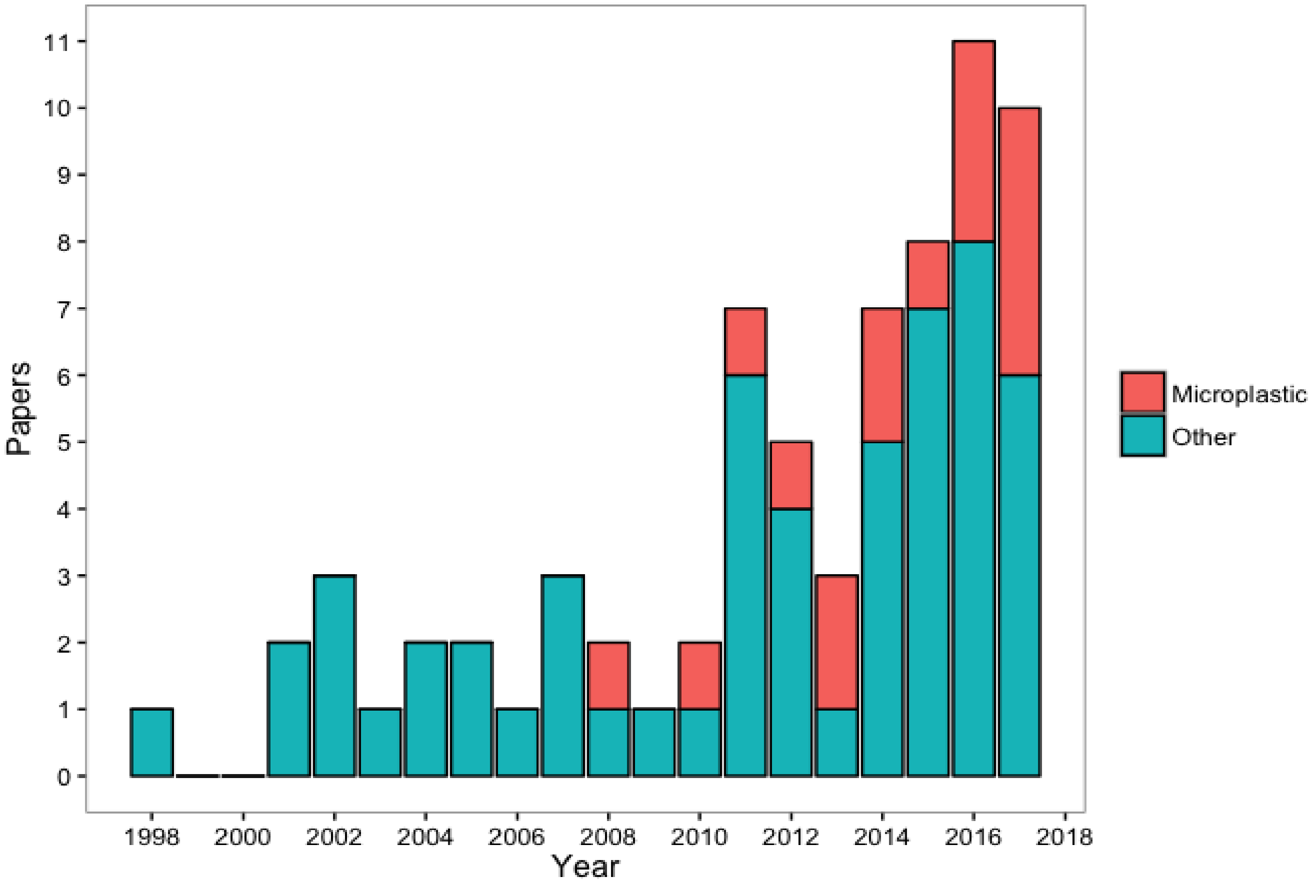

Figure 5. Inclusion of microplastics in diet analysis (either by explicitly stating it or by using a mesh size smaller than 5mm) of sea turtle studies that considered ingested plastic debris from 1998 to 2017 (n=70).

More than half of the analyzed literature (64%, n=54) did not report mesh size and did not mention whether microplastics were included. It is worth mentioning that also the two studies that found no plastic debris (Reinhold, 2015; Witzell and Schmid, 2005) did not specify the mesh size they used and, consequently, microplastics might have been excluded from their analysis. Any size restriction in the classification of ingested plastic debris should be specified, as well as the mesh size.

Four publications of the 70 analyzed explicitly exclude plastic litter smaller than 0.5 cm from their analysis. In particular, Santos et al. (2015a) and Hoarau et al. (2014) justify their choice by stating that these plastic debris may be the result of fragmentation of larger pieces inside the sea turtle. However, when possible, microplastics should be included in the study of ingested plastic debris, as more research is needed to investigate the effects of microplastic ingestion on sea turtles (Nelms et al., 2016). Recently, there has been a growing amount of reviews emphasizing the importance of microplastic as a marine pollutant (Galloway et al., 2017; Ivar Do Sul and Costa, 2014; Wright et al., 2013). Nevertheless, there have only been a few studies focusing on microplastic ingestion by sea turtles (Caron et al., 2018; Pham et al., 2017). In particular, Caron et al. (2018) have created a protocol to extract microplastic from green turtle chime, which could facilitate future studies to include microplastic in their analysis of ingested plastic debris by sea turtles.

3.4. Protocols to identify plastic debris

3.4.1. Current protocols used for the classification of ingested plastic by sea turtles

Currently, the protocol created by van Franeker et al. (2011) is the only standardized method used in sea turtle research to classify ingested debris. Besides showing how to classify ingested plastic debris, the protocol gives accurate instructions on how to perform a necropsy of a marine turtle carcass (Galgani et al., 2013). Specifically, it recommends examining the whole gastro-intestinal tract (GI). Once marine litter is extracted, the most essential information regarding its abundance should be reported, specifically the mass and volume (Galgani et al., 2013). Moreover, other information such as colour of items, incidence of litter in oesophagus, intestine and stomach should be stated as well (Galgani et al., 2013). The protocol also recommends using a 1 mm mesh size, implying that at least the larger microplastics are included in the analysis (van Franeker et al., 2011). The next step is to classify the ingested marine debris by category based on morphological characteristics. These categories differentiate plastic in "Industrial" and "User" and classify non-plastic debris as "Rubbish", "Pollutants" and "Natural non-food remains", as shown in Table 1.

Table 1. Protocol for the classification of ingested debris created by van Franeker et al. (2011) and endorsed by Galgani et al. (2013) for sea turtle research.

PLASTIC: industrial	All industrial plastic items
Pellets	Industrial plastic granules
Probably industrial	Suspected industrial
PLASTIC: user	All user plastic items
Sheet	Remains of sheet (e.g. from bag, cling-foil,…)
Thread	Threadlike materials (e.g. pieces of nylon wire,…)
Foam	Polystyrene foam, foamed soft rubber
Fragments	Broken pieces of thicker type plastic
Other	Any other, including elastics, dense rubber, cigarette filter
OTHER RUBBISH	Any other non synthetic consumer litter
Paper	Newspaper, packaging, cardboard, etc.
Kitchen food	Human food remains such as onion, beans, bacon, chicken
Other user	Litter such as processed wood, paint chips, metal
Fishing hook	Fishing hook (or pieces of it)
POLLUTANTS	Other non synthetic industrial waste
Slag/coal	Industrial oven slags or coal remains

Oil/tar	Lump of oil or tar
Paraffin/chemistry	Lump or mash of unclear paraffin, wax-like substances
Feather	Lump of feathers from excessive preening of fouled feathers
NATURAL FOOD	Various categories and it depends on the species diet
NATURAL NON-FOOD	Anything natural but which is not part of normal diet

Generally, the methodology used to classify plastic debris ingested by sea turtles is different from the one used for surveys of floating or deep-sea debris. For example, Campani et al. (2013) assessed the abundance of marine debris in the gastrointestinal tract of loggerhead turtles stranded along the coast of Western Italy; Suaria and Aliani (2014) surveyed the density of floating debris in the Mediterranean sea, including the area off the coast of Western Italy. Ideally, these two studies could be compared to see if the plastic debris found in sea turtle's stomachs have the same characteristics of the ones found at sea surface, since they were performed in the same location and time. However, their methodologies for classifying plastic litter differ, making comparison difficult. Campani et al. (2013) used the protocol by van Franeker et al. (2011) to classify marine debris, while Suaria and Aliani (2014) only differentiated between plastic fragments and Styrofoam. A standardized protocol to classify plastic debris should be employed both by biologists studying the impacts of ingested plastic debris on marine fauna and by scientists who study the movement and abundance of plastic litter in the ocean.

3.4.2. Current protocols used for the classification of plastic in ocean pollution research

There are protocols for classifying ingested plastic debris that measure non-morphological characteristics, such as chemical and elemental composition. These methods, though, are rarely used in studies that analyze the impact of plastic debris on sea turtles. They are mainly employed in research that investigates the presence and distribution of plastic litter in the ocean (Hirai et al., 2011; Martins and Sobral, 2011; Morét-Ferguson et al., 2010; Reisser et al., 2014).

3.4.2.1. Density-based protocols

A simple and inexpensive method to separate and identify the polymer composition of plastic debris is to use density measurements. There are two density-based protocols. The first one, elaborated by Reisser et al. (2014), measures the rising velocity of each piece and tests whether the ingested debris is positively buoyant in seawater. The protocol is based on the fact that negatively buoyant plastics will sink while positively buoyant plastics will float. This information can help us predict where in the water column sea turtles tend to encounter plastic debris. The protocol developed by Reisser et al. (2014) is simple and inexpensive: the only equipment needed is a graduated cylinder, seawater and a stopwatch to measure the rising velocity of each debris piece.

The second density-based protocol was first elaborated by Kolb and Kolb (1991) and then adapted by Morét-Ferguson et al. (2010). This protocol uses density measurements to determine the plastic type of the ingested debris. Only basic lab equipment is needed: a glass vial, distilled water, an automatic pipette, an analytical balance and concentrated solutions of calcium or strontium chloride. The protocol is based on the fact that each virgin plastic type has a specific density range. However, the specific density of each plastic piece changes at sea due to erosion and overgrowth by micro- and macro-organisms (biofouling) (Hidalgo-Ruz et al., 2013). Biofouling tends to decrease buoyancy and thus contributes to plastic debris sinking, while erosion increases buoyancy (Cozar et al., 2014; Hidalgo-Ruz et al., 2013).

3.4.2.2. Elemental analysis

Morét-Ferguson et al. (2010) integrated elemental analysis to their density-based protocol to determine with higher accuracy the virgin plastic type of marine debris. By looking at the carbon, hydrogen and nitrogen (CHN) content, they were able to determine the correct polymer type when the density was different than the one of the virgin plastic type. CHN analysis is a simple and informative method that can easily be integrated with density sample analysis to determine the polymer composition of plastic debris, but its big disadvantage is that it is a destructive technique, as it involves combustion of the sample (Niaounakis, 2017). The equipment required for this analysis is a CHN analyzer.

3.4.2.3. Infrared and Raman Spectroscopy

Infrared spectroscopy allows a precise identification of plastic polymer fragments according to their typical IR spectra (Rocha-Santos and Duarte, 2015; Wesch et al., 2016; Zobkov and Esiukova, 2018). There are different types of infrared spectroscopy: infrared spectroscopy (IR), Fourier transform infrared spectroscopy (FTIR) and NIR spectroscopy (Niaounakis, 2017). FTIR is particularly effective for the analysis of microplastics. This method is highly reliable and reproducible, however it requires expensive equipment: a FT-IR linked to a microscope (Zobkov and Esiukova, 2018). Raman spectroscopy is another efficient chemical analysis (Zobkov and Esiukova, 2018). An important advantage of the Raman spectroscopy methodology is that no sample preparation is needed and it is non-destructive (Niaounakis, 2017).

Nevertheless, this technique requires sophisticated and expensive equipment (Niaounakis, 2017).

3.5. Decision-making tree: which protocol to use?

A variety of methods exist to analyze ingested plastic debris. Each plastic item has multiple characteristics, morphological and physical-chemical. Examining all characteristics would be considerably expensive and time consuming because it would imply using several different methods. What kind of information can be obtained from each method? How to choose which method to use? Which is fit to purpose?

The choice of methodology should depend on the focus of each study (as summarized in Figure 6) and it should be designed in a way that it answers the research question. Nevertheless, we advise that a basic quantification (mass, volume and %FO) of plastic debris should be done in any study that finds plastic litter ingested by sea turtles, no matter the focus. Several other papers recommend reporting these basic parameters (Clukey et al., 2017; Fossi et al., 2018; Nelms et al., 2016; Provencher et al., 2017). The impact plastic debris ingestion has on certain sea turtle species and in certain geographical areas is still understudied (Nelms et al., 2016; Schuyler et al., 2014a). Hence, information on plastic debris vs. sea turtle interactions should be maximized within the capacity of each study.

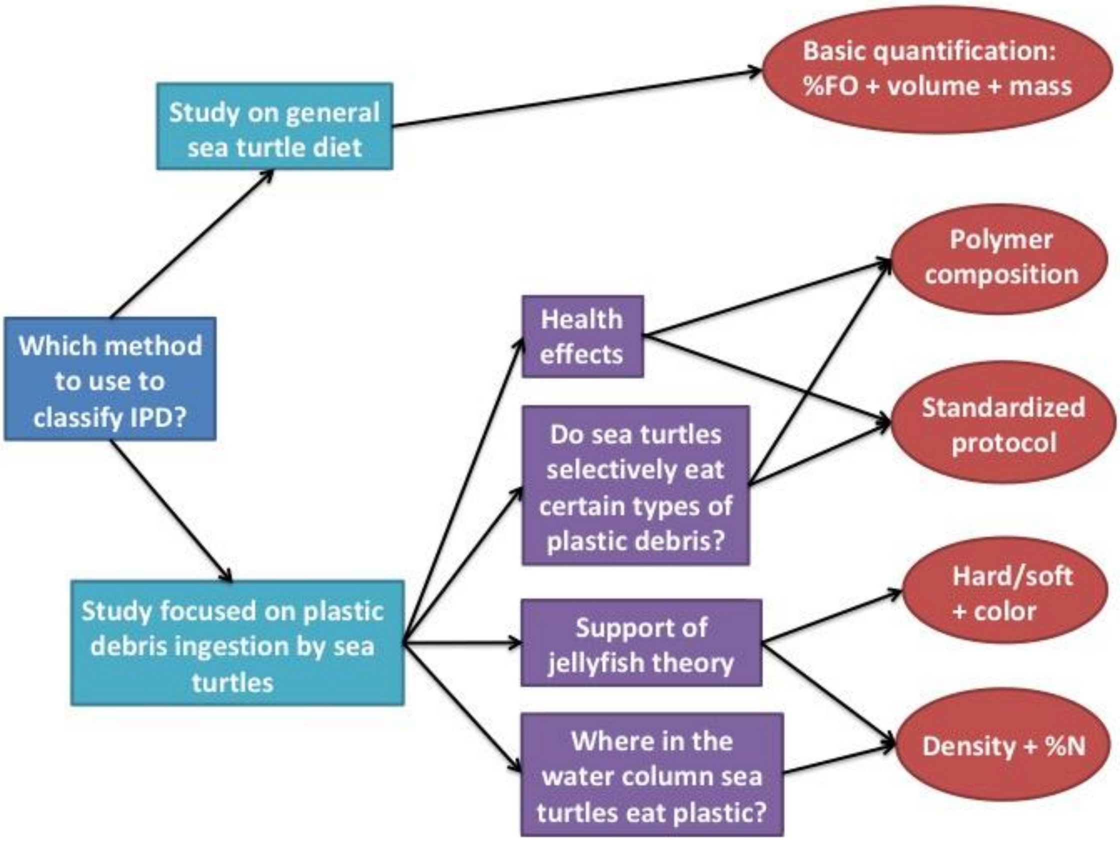

Figure 6. Diagram showing the various steps that lead to a decision on which method to use to classify ingestion of plastic debris (IPD) by sea turtles. The standardized protocol is the one created by van Franeker et al. (2011).

Contrarily, for studies that concentrate on plastic debris ingestion by sea turtles, IPD quantification is not sufficient and it should be followed by a classification of IPD. The classification method should differ depending on the study purpose. For example, studies with the aim to investigate the health effects of plastic debris ingestion on sea turtles should analyze the polymer composition of IPD. In order to examine the most common lethal effects, such as intestinal blockage and perforation, a basic quantification of plastic debris is enough. However, identifying the polymer composition of ingested plastic debris can help us detect other sub-lethal effects, such as chemical contamination. Secondary contamination by plastic consumption has been shown in several marine taxa but it remains poorly understood in sea turtles (Clukey et al., 2018; Gall and Thompson, 2015; Galloway et al., 2017; Nelms et al., 2016; Rios et al., 2007) . The identification of the IPD polymers will permit distinguishing between toxic chemicals that have sorbed to plastic debris in the marine environment and pollutants that were introduced during the manufacturing process (Lithner et al., 2011; Rios et al., 2007).

It is still unclear why sea turtles eat plastic (Di Beneditto and Awabdi, 2014; Nelms et al., 2016). Several possible explanations have been suggested: (i) the jellyfish hypothesis, which states that plastic bags resemble jellyfish, a typical prey item, and thus sea turtles, which have been proven to feed visually (Schuyler et al., 2012), eat this kind of plastic (white/transparent sheet-like plastic) because they confuse it for their natural prey (Mrosovsky, 1981) (ii) sea turtles ingest plastic debris because it is mixed with their natural food (Di Beneditto and Awabdi, 2014; Russell et al., 2011; Witherington, 2002); (iii) since sea turtles are opportunistic feeders, they eat what they encounter the most in their environment (Matiddi et al., 2017; Pham et al., 2017; Tourinho et al., 2010).

If the purpose of a study is to verify the jellyfish hypothesis, we recommend classifying IPD according to texture (hard/soft) and color. We also recommend measuring the density of IPD, to verify that the ingestion happened in the water column, where normally sea turtles feed on jellyfish (Fukuoka et al., 2016; Nelms et al., 2016). We found that of the 68 papers we reviewed that considered and found IPD, 25% (n=18) support the jellyfish hypothesis. There is an increase in studies that support the jellyfish hypothesis in 2014, with a peak in 2016 (8%), as shown in Figure 7. This growth could be explained by the publication of two studies that directly tested debris selectivity by visual cues (Schuyler et al., 2014b, 2012).

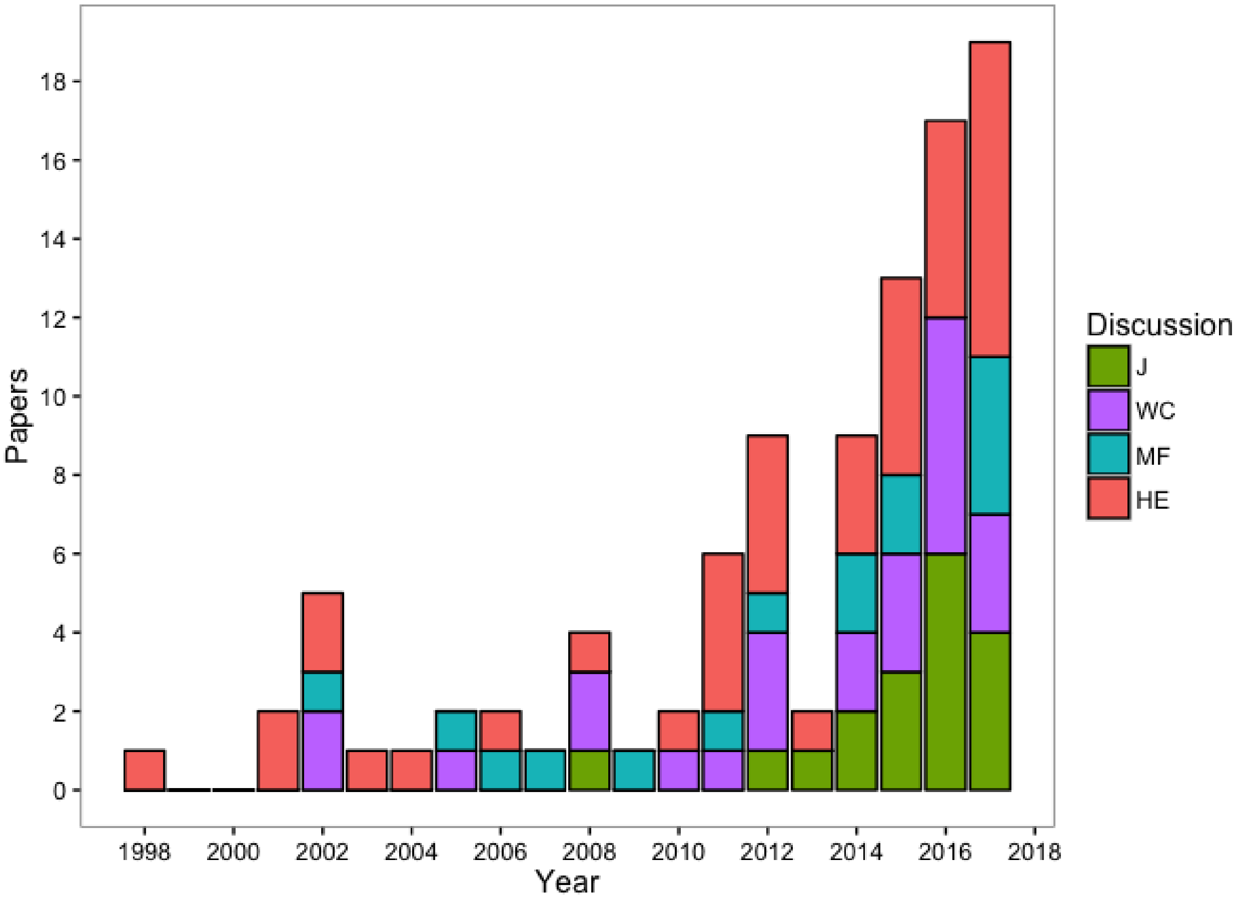

Figure 7. Tendency of published studies on sea turtles diet from 1998 to 2017 that consider and found ingested plastic debris to address "where in the water column sea turtles eat plastic debris" (WC), "plastic debris effects on sea turtle health" (HE) and to support two hypothesis for why sea turtles eat plastic: "plastic debris is eaten because it looks like jellyfish" (J), "plastic debris is eaten because it is mixed with natural food" (MF).

Many studies discuss where in the water column sea turtles eat plastic debris (e.g., Fukuoka et al., 2016; Ryan et al., 2016; Santos et al., 2016; Vélez-Rubio et al., 2017). The likelihood of exposure to plastic debris differs according to whether a turtle feeds pealgically or benthically, which depends on the species and life stage (Bolten, 2003; Nelms et al., 2016). Moreover, plastic debris can be found floating at the sea surface, settled at the bottom or adrift in the middle of the water column (Engler, 2012; Galloway et al., 2017; Kaiser et al., 2017). Knowing where in the water column sea turtles eat plastic debris is key to find solutions to limit the impacts of ocean plastic pollution on sea turtles.

If one of the study objectives is to know where in the water column sea turtles eat plastic debris, we recommend measuring the specific density of each piece and calculate its CHN content. By knowing the density we can infer where in the water column sea turtles ingest a certain type of plastic debris. Furthermore, by conducting a CHN analysis we can obtain the %N, which indicates biofouling. However, most studies solely examine the physical appearance of IPD. In our literature review we found that of the 24 publications that discuss where in the water column sea turtles eat plastic debris, only five test buoyancy (Fig 3).

It is still not clear whether sea turtles selectively eat a certain type of plastic or if they eat the most common plastic debris they find in their environment (Camedda et al., 2014; Nelms et al., 2016). Knowing whether selectivity by sea turtles exists and tracing back the original plastic product of the ingested debris would contribute to finding solutions for the mitigation of plastic pollution impacts on sea turtles (Eagle et al., 2016). Classifying IPD by morphological type, following the standardized protocol by van Franeker et al. (2011), can contribute to the identification of the original plastic product of the ingested debris. Moreover, especially for microplastics, it is preferable to perform an additional analysis with spectroscopy to identify the polymer composition. Particularly for resin types (such as PET, number 1) that are mostly used for the same type of object (in this case, plastic bottles) this analysis can be particularly informative on the original source of debris. In the literature we reviewed, only one study (Pham et al., 2017) used spectroscopy to identify plastic debris.

3.6. Recommendations for future research

In order to have a better knowledge of the causes that lead sea turtles to eat plastic debris, we need more research that incorporates plastic debris analysis with diet analysis. Studying sea turtles feeding dietary preferences can offer insight on whether sea turtles select certain types of plastic debris over others (Santos et al., 2015b). Even though we have a general idea of the diet of each species at different life stages (Bolten, 2003), it has been shown that sea turtle diet varies considerably depending on the characteristics of the foraging area and on the food availability (Gama et al., 2016; Vélez-Rubio et al., 2016). For example, green turtles in estuarine areas have a more generalist foraging ecology than green turtles feeding in reefs (Nagaoka et al., 2012; Santos et al., 2015b). This foraging behavior coupled with the fact that estuarine environments tend to be particularly polluted can considerably increase sea turtle vulnerability to plastic debris ingestion (González Carman et al., 2014; Santos et al., 2015b).

Diet analysis may not be sufficient to understand what happens when a sea turtle encounters and eats a piece of plastic. For example, it is difficult to determine solely through a dietary analysis whether sea turtles eat plastic debris because it is mixed with their natural food. Research more focused on the foraging ecology and feeding behavior of sea turtles could contribute to the comprehension of the causes of plastic debris ingestion. In particular, studies implementing observation methods such as animal-borne video camera (Fukuoka et al., 2016) and direct observation (Reisser et al., 2013) are ideal to investigate how sea turtles react when they encounter plastic debris. More studies of this kind will enable us to understand whether sea turtles react differently to different types of plastic debris.

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5. Supplementary material

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CHAPTER III

Conclusions

1. Conclusão

A ingestão de detrito plástico por tartarugas-marinhas foi bem documentada nas últimas décadas (Gall and Thompson, 2015; Nelms et al., 2016). No entanto, a falta de um método padrão de classificação dos detritos plásticos ingeridos reduz nossa habilidade de comparar estudos (Provencher et al., 2017). Recentemente, graças a vários estudos destacando a importância da padronização (Fossi et al., 2018; Galgani et al., 2013; Nelms et al., 2016; Provencher et al., 2017) um crescente número de publicações em ingestão de tartarugas-marinhas vêm utilizando o protocolo proposto por van Franeker et al. (2011) para classificar o detrito plástico ingerido (e.g., Camedda et al., 2014; Pham et al., 2017; Vélez-Rubio et al., 2017). Não obstante, este protocolo somente examina as características morfológicas do detrito plástico, como cor, textura, forma e massa; também a maioria dos estudos de que tratamos somente analisam a morfologia de detritos plásticos ingeridos. Medir as propriedades físicas do detrito plástico, como sua flutuabilidade e composição de polímeros, irá aprimorar nossa habilidade de compreender as causas da ingestão de detrito plástico por tartarugas-marinhas. A medição destas propriedades requere o uso de protocolos que são amplamente utilizados na pesquisa de poluição plástica (Morét-Ferguson et al., 2010; Zobkov and Esiukova, 2018), mas não na pesquisa de tartarugas-marinhas. Com o objetivo de aproximar estas duas áreas de pesquisa, nós recomendamos o uso da árvore de decisões que criamos para estudos futuros de ingestão de detrito por tartarugas-marinhas. Implementar métodos que classificam plástico ingerido pertinente ao objetivo do estudo nos trará mais perto da compreensão de relações entre o plástico e as tartarugas-marinhas.

2. Conclusions

Plastic debris ingestion by sea turtles has been well documented in the last few decades (Gall and Thompson, 2015; Nelms et al., 2016). However, a lack of standardization in the methods used to classify ingested plastic debris has reduced our ability to compare studies (Provencher et al., 2017). Recently, thanks to various studies emphasizing the importance of standardization (Fossi et al., 2018; Galgani et al., 2013; Nelms et al., 2016; Provencher et al., 2017), a growing number of publications on sea turtle plastic ingestion have been using the protocol by van Franeker et al. (2011) to classify ingested plastic debris (e.g., Camedda et al., 2014; Pham et al., 2017; Vélez-Rubio et al., 2017). Nevertheless, this protocol only examines the morphological characteristics of plastic debris, such as color, texture, shape and mass; also the majority of the studies that do not use this protocol solely analyses the morphology of ingested plastic debris. Measuring the physical properties of plastic debris, such as buoyancy and polymer composition, will improve our ability to understand the causes of sea turtle plastic ingestion. The measurement of these properties require the use of protocols that are widely used in plastic pollution research (Morét-Ferguson et al., 2010; Zobkov and Esiukova, 2018), but not so in sea turtle research. With the aim of bridging the gap between these two research areas, we recommend using the decision-making tree we created for future studies on sea turtle debris ingestion. Implementing methods to classify ingested plastic debris fit to the study purpose will bring us closer to understand sea turtle – plastic interactions.

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