UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

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Understanding the effects of plastic pollution on sea turtles: a global review.

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Understanding the effects of plastic pollution on sea turtles: a global review. Fernando Andrés Cisneros Bejarano

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RESUMEN

La contaminación plástica se ha convertido en una de las mayores amenazas para los ecosistemas marinos y la salud de las tortugas marinas en todo el mundo. La ingestión y el enredo de plástico amenazan a las siete especies de tortugas marinas a nivel mundial(*Chelonia mydas*, *Caretta caretta*, *Eretmochelys imbricata*, *Dermochelys coriacea*, *Lepidochelys kempii*, *Lepidochelys olivacea* y *Natator depressus*). El objetivo de este estudio fue determinar los impactos de la contaminación plástica en la salud de las tortugas marinas. Se ha informado de enredos e ingestión de plástico en todas las etapas de la vida, y la mayoría de los estudios se han realizado con individuos muertos e individuos juveniles. Se están describiendo y utilizando nuevos métodos con tortugas que están vivas. Se realizó una revisión sistemática para encontrar literatura relevante en la que se encuentre ingestión o enredo de plástico en las siete especies de tortugas marinas. Cuarenta y dos artículos cumplieron con los criterios de inclusión. La ubicación de donde se están muestreando las tortugas para determinar dónde ha habido más estudios y dónde ha habido más contaminación plástica. La ingestión de colores plásticos se establece para determinar qué tipo de color son más propensos a ser ingeridos, siendo el color más ingerido: blanco/transparente. Se realizó una puntuación de prioridad para observar si existen diferencias significativas, realizada con la prueba de Kolmogorov-Smirnov y un valor de p <0,0001, entre la evidencia encontrada sobre entrelazamiento vs ingestión de plástico y la puntuación de amenaza de entrelazamiento vs ingestión en las especies de tortugas marinas. La contaminación plástica continúa propagándose en el ecosistema marino y se deben tomar medidas para desarrollar técnicas para extraer el plástico que ha ingresado al tracto gastrointestinal.

Palabras clave: contaminación plástica, tortugas marinas, enredo, ingestión de plástico, revisión sistemática, color, ubicación, salud.

ABSTRACT

Plastic pollution has become one of the biggest threats to marine ecosystems and to the health of sea turtles across the world. Plastic ingestion and entanglement are threatening the seven species of sea turtles around the world (*Chelonia mydas*, *Caretta caretta*, *Eretmochelys imbricata*, *Dermochelys coriacea*, *Lepidochelys kempii*, *Lepidochelys olivacea*, and *Natator depressus*). Entanglement and plastic ingestion have been reported at all life stages, with most of the studies being done in dead individuals and in juveniles. However, there are also new methods being developed and used in living sea turtles. The objective of this study was to determine the impacts of plastic pollution on the health of sea turtles. A systematic review was done to find relevant literature in which plastic ingestion or entanglement was identified in the seven species of sea turtles. Forty-two papers met the criteria inclusion. The location of where the turtles were sampled was used to determine where there have been more studies and where has been more plastic pollution. Plastic color ingestion was considered to determine what type of color is more prone to be ingested, white/transparent plastics being the most ingested color. A priority score was made to observe if there were significance differences, done with Kolmogorov-Smirnov-test and a p value of \leq 0,0001, between the evidence found on entanglement vs plastic ingestion and the threaten score of entanglement vs ingestion in sea turtles species. Plastic pollution continues to spread in the marine ecosystem and action needs to be taken in order to develop techniques to extract plastic that has entered the gastrointestinal tract of turtles.

Key words: plastic pollution, sea turtles, entanglement, plastic ingestion, systematic review, color, location, health.

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INTRODUCTION

Plastics are one of the most common and persistent pollutants in coastal and marine environments across the world (Caron et al. 2018). Plastic can be found from the polar regions to the equator, from shorelines to highly populated coastlines, from the coastline far too and down into the deep sea (Wang et al. 2016). It has become the most ubiquitous and ongoing pollutant found in marine environments. Plastic debris and discarded or lost nylon fishing gear in particular make up a significant portion of plastic pollution affecting the world's oceans (Barreiros and Raykov 2014). Plastic debris in general is abundant in marine environments (Santos et al. 2015). The ocean is polluted with more than 6 million metric tons of plastic debris per year (Franzen-Klein et al. 2020), with 80 % of plastic roots coming from land sources (Schuyler et al. 2014). Human dependency on plastic has caused an increase in the production of this material and as a result has inadvertently impacted marine species (Abreo et al. 2016). Plastic pollutants are divided into two categories: macroplastics (> 5 mm) and microplastics (< 5 mm) (Caron et al. 2018). Most of the plastic found in the marine environment is single-use plastic (Angelo et al. 2019). On the ocean's surface, the estimated amount of small floating plastic is 10 million times higher in the subtropical accumulation regions than in the Southern Ocean (van Sebille et al. 2019).

Over 900 marine species have been reported to have interacted with plastic (Senko et al. 2020). Air-breathing marine megafauna (sea birds, marine mammals, and sea turtles) are the taxa most commonly reported to interact with plastic pollutants (Senko et al. 2020), with 86% of marine turtles, 44% of sea birds, and 43% of marine mammals affected (López-Martínez et al. 2021). Plastic pollution can cause lethal individual-level effects to marine megafauna such as entanglement or ingestion, but can also have sub-lethal effects that may influence resource acquisition, health, and reproductive output (Senko et al. 2020). Entanglement affects marine animals' health by causing physical injuries and illness (including lacerations, constriction, severe sclerosis, loss of limbs, and difficulty breathing if the airway) (Senko et al. 2020), as well as physiological stress (inhibiting diving and increasing hydrodynamic drag). In sea turtles, the levels of corticosterone while being entangled have been studied (Hunt et al. 2016), and have been found to reduce mobility (López-Martínez et al. 2021). However, one of the predominant ways in which plastic ingestion affects the health of marine animals is by causing issues in the gastrointestinal tract (such as ulcerations, perforations, and laceration of the larynx that can result in chronic infection, peritonitis, gastrointestinal motility issues, septicemia, and mortality, dietary dilution, exposure to contaminants associated with plastic pollution, and reduced mobility) (Senko et al. 2020).

Marine debris ingestion has been reported in all seven species of sea turtle (Franzen-Klein et al. 2020). These species are: *Chelonia mydas* (green sea turtle), *Caretta caretta* (loggerhead sea turtle), *Eretmochelys imbricata* (hawksbill sea turtle), *Dermochelys coriacea* (leatherback sea turtle), *Lepidochelys kempii* (Kemp's Ridley Sea turtle), *Lepidochelys olivacea* (Olive Ridley Sea turtle), and *Natator depressus* (flatback sea turtle). All of these species have been listed on the International Union of Conservation of Nature (IUCN) list: *C. mydas* is listed endangered (Seminoff 2004), *C. caretta* is listed as vulnerable, (Casale & Tucker, 2017), *E. imbricata* is listed as critically endangered (Mortimer and Donnelly, M. 2008), *D. coriacea* is listed as vulnerable (Wallace, Tiwari, & Girondot, 2013), *L. kempii* is listed as critically endangered (Wibbels & Bevan, 2019), *L. olivacea* is listed as vulnerable (Abreu-Grobois 2008), and *N. depressus* is listed as data deficient (IUCN, 1996). Sea turtles are threatened at all their life stages (from nesting on beaches to life at sea) due to human impacts (Tomás et al. 2002). Sea turtles are prone to eat plastic (Carr 1987). Turtles that live in oceanic or coastal environments might encounter different densities of marine debris that may result in ingestion or entanglement (Schuyler et al. 2014). Among the seven species of sea turtles, some are carnivorous species (adult loggerhead and Kemp's ridley), herbivores (green), omnivores (hatchlings loggerhead and green, hawksbill, flatback, and Olive ridley), and gelatinovores (leatherback) (Schuyler et al. 2014). The herbivores, such as the green turtle are more susceptible to ingestion of marine debris (Caron et al. 2018).

Plastic can affect sea turtles through entanglement or ingestion, which can result in drowning, perforation, obstruction of the gastrointestinal system, reduction of nutrient absorption, absorption of toxic plasticizers, and suppression of the immune system (Wedemeyer-Strombel et al. 2015). In sea turtles, marine debris has been reported mostly in juveniles (Duncan et al. 2019), but can also affect the properties of the nest and consequently shift hatchling sex ratios, prevent the hatchlings from leaving the egg chamber, act as a barrier in the sand column, or present an obstacle to the hatchling while trying to reach the ocean (Aguilera et al. 2018). Most of the studies that involve plastic ingestion by sea turtles are done with dead individuals, in which necropsies are made and the gastrointestinal track is extracted in search of plastic ingestion (Clukey et al. 2017, Darmon et al. 2017, Rizzi et al. 2019). However, there are also new methods to identify plastic ingestion on sea turtles (Hoarau et al. 2014, Gündoğdu et al. 2019, Franzen-Klein et al. 2020, Biagi et al. 2021). In one of the methods, the sea turtles undergo an endoscopy in order to identify plastic in the gastrointestinal tract. This method is implemented on living sea turtles (Muñoz-Pérez et al., in prep).

The purpose of this study is to determine the impacts of plastic pollution on the health of the seven species of sea turtle using relevant literature that mentions the impacts of plastic on the health of sea turtles. The specific goals for this study are to (1) identify papers that reveal the impacts of plastic pollution and health, (2) within those studies, identify which species of sea turtle is more susceptible to eat plastic or be entangled, (3) to classify what color of plastic is more likely to be consumed by the seven species of sea turtles, and (4) identify any significance differences between entanglement and plastic ingestion. Within the third specific goal, the methods for plastic extraction on both dead and living sea turtles will be discussed. In summary, a systematic review of the literature was completed, which yielded 42 papers considered relevant to the research question. It is important to study the effects of plastic pollution on the seven species of sea turtle due to the fact that marine plastic pollution occurs on a global scale; in order to understand how to best protect sea turtles, the effects of plastic pollution on their health must be understood first.

METHODS

Literature review

This present study focuses on the examination of relevant literature that identify the impact of plastic on all seven species of sea turtles, including both dead and living individuals.

The literature research was completed between August and October of 2021, including articles from 1987 to 2021, which were identified using the database of Scopus database for the first three searches and Google Scholar for a fourth and a complementary search. The articles were chosen according to both their relevance in the context of this study as well as whether they had free or open access. The protocol for article selection was made using the research question and the PIO (Population, Intervention, and Output). The terms for Population were *sea AND turtle* OR "dermochelys coriacea" OR "caretta caretta" OR "chelonia mydas" OR "natator depressus" OR "eretmochelys imbricata" OR "lepidochelys kempii" OR "lepidochelys olivacea".* The terms for Intervention were: *microplastic* OR plastic* OR "plastic particle*" OR "plastic pollution" OR "marine pollution" OR "microplastic pollution" OR "marine debris" OR "entanglement".* Finally, the terms for Output were: *"ingestion" OR "effects" AND "impact" OR "consequences" OR "effects" AND "health*"*. The terms "OR"/"AND" ensure more specificity with the search, avoiding other topics or other animals. Also, the asterisk (*) was used to certain terms that represent any group of character, including the absence of these; the quotation marks (") were used to define a literal search for specific words.

The terms used for the first literature search in Scopus were the following: *microplastic* OR plastic* OR "plastic particle*" OR "plastic pollution" OR "marine pollution" OR "microplastic pollution" OR "marine debris" OR entanglement AND * OR "ingestion" OR "effects" AND "impact" OR "consequences" OR "effects" AND sea AND turtle* OR*

"dermochelys coriacea" OR "caretta caretta" OR "chelonia mydas" OR "natator depressus" OR "eretmochelys imbricata" OR "lepidochelys kempii" OR "lepidochelys olivacea".

For the second search in Scopus, a new term was added: *microplastic* OR plastic* OR "plastic particle*" OR "plastic pollution" OR "marine pollution" OR "microplastic pollution" OR "marine debris" OR "entanglement" OR "ingestion" OR "effects" AND "impact" OR "consequences" OR "effects" AND "health*" AND "sea turtle* " OR "marine turtles*" OR "dermochelys coriacea" OR "caretta caretta*" OR "chelonia mydas*" OR "natator depressus*" OR "eretmochelys imbricata*" OR "lepidochelys kempii*" OR "lepidochelys olivacea*"*.

One final search was made in Scopus to determine entanglement within the seven species of sea turtle using the following terms: entanglement* AND "impact" OR "consequences" OR "effects" AND "health*" AND "sea turtle*" OR "marine turtles*" OR "dermochelys coriacea" OR "caretta caretta*" OR "chelonia mydas*" OR "natator depressus*" OR "eretmochelys imbricata*" OR "lepidochelys kempii*" OR "lepidochelys olivacea*".

For the fourth complementary search, Google Scholar was used, and the terms used for the search were: *("plastic" OR "microplastic" OR "plastics" OR "plastic pollution" OR "marine debris" OR "fishing net*" OR "ghost fishing") AND ("impact" OR "entanglement" OR "ingestion" OR "effects" OR "consequences" OR "health effects") AND ("sea turtle").* This information can also be found in Appendix A and Appendix B.

The criteria for an article's inclusion for the Scopus searches and Google Scholar were the following: only published papers, no books or book chapters, articles published only in English, methods implemented on sea turtles (either dead or alive) to analyze the impacts of plastic on these animals, articles published since the first record of plastic pollution in sea turtles, and articles in which the content do not make references to the three aspects englobing the question: population, intervention, and output. However, for the Google Scholar article

selection, the range for the article search was from 2019-2021 due to the fact that studies with endoscopy procedures on living sea turtles were found within this period.

Once the results were obtained, an initial screening of the titles of 372 articles was carried out, followed by a screening of the titles and abstracts of 241 articles. From these articles, 131 were given a major screening which included a full reading of the article, leaving a total of 42 articles. This process can be seen in Figure 1. The papers selected were focused on the impacts of plastic pollution on sea turtles. Most of the papers describe how plastic ingestion was found in dead sea turtles, affecting and blocking their esophagus, stomach, large and small intestine. However, new methods with live turtles are being used to determine their health status. These methods allow plastic to be removed without harming the turtles.

Priority scoring

In the 42 papers chosen, the seven species of sea turtle were described at least one time. The species were given a distribution score (S^D): invasive (S^D = 0), migratory (S^D = 1), native $(S^D = 2)$ or endemic $(S^D = 3)$. Most of the species considered in this study can be found around the world, except for flatback and Kemp's Ridley turtles. The IUCN Red List status for each species was recovered from the IUCN database (https://www.iucnredlist.org/) to generate a conservation score (S^C): data deficient, not evaluated, and least concern (S^C = 1), near threatened and vulnerable ($S^C = 2$) or endangered and critically endangered ($S^C = 3$). To determine if the plastic was entangled (S^{EL}) or ingested (S^{IL}) by the sea turtles, a literature review was made, finding 42 papers that showed this harm. The literature was organized into three categories in which the species interaction with plastic was described. The categories are the following: No evidence: *No current evidence of the effects of plastic ingestion* (S^{EL} or S^{IL}) = 1); Moderate: *There is evidence that demonstrates that the species had some interactions with marine plastics which resulted in non-lethal effects* (S^{EL} or $S^{IL} = 2$); Major: *There are multiple pieces of evidence that demonstrate the species had major interactions with marine* *plastics that resulted in severe injury or even the death of the individual* (S^{EL} or $S^{IL} = 3$) (Jones et al. 2021). To calculate the priority species at high threat from marine plastic entanglement and ingestion, the following equations were developed:

$$
E = S^{D} \times S^{C} \times S^{EL}
$$

$$
I = S^{D} \times S^{C} \times S^{IL}
$$

Statistical analysis

All results were generated in Microsoft Excel, each one using different formulas and ideas for the figures; and all statistical analysis used in all the results. All graphics were generated in Microsoft Excel

A Kolmogorov-Smirnov unpaired test was conducted to determine if there are significant differences between the number of turtles with plastic ingestion and no plastic ingestion, and the turtles found entangled and the turtles found with no entanglement. This test is an unpaired and nonparametric, which compares cumulative distribution.

RESULTS

Systematic review

A total of 372 documents (including articles and book chapters) were found in the literature search using Scopus and Google Scholar. However, only 42 papers met the inclusion criteria, the details of which can be found in Table 1. The selection process can be seen in Figure 1 and the 42 articles can be found in Appendix C, where the following details are shown: title, author, year of publication, and other details.

Figure 1. Filters used for the article search using the research question.

The papers that were chosen reported plastic ingestion in one or multiple of the seven species of sea turtle and identify how the sea turtle health was affected by this plastic ingestion

from the year of publication (Figure 2). Figure 2 shows the cumulative number of studies, which grows in slope as it reaches the 42 papers.

Figure 2. Cumulative number of studies per year of plastic ingestion by sea turtles.

Of the 42 papers that were chosen, the first one that mentions plastic pollution in sea turtles is from 1987. From that year until the present year (2021), more studies continue to be published mentioning the impacts of plastic contamination on sea turtles' health. Numerous papers are being published every year regarding the development of different methods to identify the effects of plastic on the seven species of sea turtles, including macro and microplastics.

Location for the turtles that were sampled

Figure 3. Locations per country for the sea turtles found in the systematic review.

In Figure 3, all the locations from which the turtles were sampled are listed. Most of the studies took place in the U.S.A. (eight studies in Florida, two in North Carolina, one in Georgia, and one in Massachusetts). Additionally, there are two global studies made by (Duncan et al. 2017) and in (Schuyler et al. 2014).

Table 1. All of the 42 papers found for the systematic review, which includes the authors, number of turtles per study, which species were used in the study, and the percentage of turtles that have ingested plastic or had entanglement with plastic per study. Based on the paper published by Schuyler et al. (2014).

Schuyler et al. (2014) presented a table which described a systematic study of plastic ingestion by sea turtles, but the data used was only collected until 2012. So, in Table 1, a similar table was made, based on Schuyler et al (2014), but instead using data collected up until 2021. As can be seen in Table 1, most of the papers represent at least one species of sea turtle that had ingested plastic or found entangled. However, eighteen of these studies: (1. Aguilera et al. (2018), 2. Barreiro's & Raykov (2014), 7. Carr (1987), 8. Casale et al. (2010), 11. Darmon et al. (2017), 13. De Carvalho-Souza et al. (2016), 16. Duncan et al. (2017), 19. Franzen-Klein et al. (2020), 20. Gündogdu et al. (2019), 22. (Hunt et al. 2016), 23. (Jensen et al. 2013), 27. (Miguel et al. 2020) 29. Nelms et al. (2016), 30. Oliveira et al. (2020), 34. (Senko et al. 2020),

35. Schuyler et al. (2014), 37. (Snoddy et al. 2009), and 41. (Wilcox et al. 2013)) had no data and were a different type of study.

In Aguilera et al. (2018), for example, while the paper does mention a total number of individuals (232 post-hatching loggerhead sea turtles), the study itself focuses only on marine debris distribution on various beaches on Boa Vista Island. Carr (1987) briefly described the ecology and survival of sea turtles but does not specifically mention any individuals with plastic ingestion. Darmon et al. (2017) mentions 477 individuals from all species of sea turtles but does not refer to any impacts of plastic pollution. De Carvalho-Souza et al. (2016) described the first *in situ* consumption of synthetic rope fragments in green sea turtles. Franzen-Klein et al. (2020) discussed endoscopy and other methods used on living sea turtles. Gündogdu et al. (2019) described potential interactions of green turtles during a nesting period on a very polluted beach located on the coast of Turkey. Nelms et al. (2016) discussed what the world should do to protect sea turtles, since all seven species have been listed on the IUCN Red List. Oliveira et al. (2020) described the digestive tracts of sea turtles and how they work. Schuyler et al. (2014), of which some data and analysis are included in this present study, is a global analysis that describes articles published from 1985 up to 2012. Duncan et al. (2017) is the basis for Table 1. Barreiros & Raykov (2014), Casale et al. (2010), Hunt et al. (2016), Jensen et al. (2013), Miguel et al. (2020), Senko et al. (2020), Snoddy et al. (2009), and Wilcox et al. (2013) are studies that focused on plastic entanglements in the seven species of sea turtle.

The 24 remaining papers presented studies with more than 10 individuals studied, with some sea turtles presenting a percentage of plastic ingestion. In those studies, all seven species were mentioned at least one time. The paper of Duncan et al. (2019), mentioned that all seven sea turtles species (102) had ingested plastic (100%). Most of the studies have more than \geq 30% of individuals with plastic ingestion. The lowest percentage of plastic ingestion found in the 42 papers was 34 % by Mrosovsky et al. (2009) and included 408 leatherback turtles. Out of these 42 studies, 22 studies mentioned loggerhead turtles, 20 studies mentioned green turtles, 10 studies mentioned leatherback turtles, nine studies mentioned olive ridley, six studies mentioned hawksbill turtles, five studies mentioned Kemp's ridley, and four studies mentioned flatback sea turtles.

Figure 4. Stacked column of sea turtles with and without plastic ingestion for the 42 papers that were chosen for the systematic review.

Figure 5. Stacked column of sea turtles that had and had not experienced plastic entanglement in the 42 papers that were chosen for the systematic review.

In Figure 4, the stacked column shows the number of sea turtles that had ingested plastic and had no plastic ingestion on the 42 papers that were chosen for the systematic review. Only 30 papers described sea turtle individuals. As mentioned before, 18 papers did not present data of individuals with plastic ingestion. The remaining 24 papers presented individual plastic ingestion data. From those 24, 17 presented more than 50% of sea turtles that had ingested plastic and 14 presented less than 50% of individuals with plastic pollution. In one paper, 100% of the sea turtles presented plastic ingestion and included all seven species. However, in Figure 5, the stacked column shows the number of sea turtles that were found entangled on the 42 papers that were chosen.

One Kolmogorov-Smirnov unpaired test was conducted on the data shown in Figure 4 to determine if there are significant difference between turtles with plastic ingestion and turtles with no plastic ingestion. The *p* value was 0.1344, meaning that there are no significant differences between turtles with plastic ingestion and turtles with no plastic ingestion. The *p* value was obtained from $p < 0.05$, and the result per the Kolmogorov-Smirnov test (D) was 0.3000. Additionally, another Kolmogorov-Smirnov unpaired test was conducted on the data shown in Figure 5, to determine if there are significant differences between turtles found entangled and turtles with no entanglement. The *p* value was < 0.0001, meaning that there are significant differences between turtles with entanglement and turtles with no entanglement using *p* value < 0.05 , and the Kolmogorov-Smirnov (D) = 0.8667.

Figure 6. Total number of sea turtles found in the 42 papers.

In Figure 6, a total of 8821 sea turtles was found to be distributed throughout the seven species represented in the 42 studies chosen for this review. Out of this total, 1390 individuals (from the seven species) had ingested plastic and 7431 did not ingest plastic. The species that presented the most plastic ingested was the green turtle (*C. mydas*), with a total of 1357 (621 ingested plastic and 736 did not) individuals that included both juveniles and adults. Flatback (*N. depressus*) had four individuals identified, with one individual having ingested plastic. Loggerhead presented 6725 individuals, leatherback presented 484 individuals, Olive ridley presented 190 individuals, Hawksbill presented 40 individuals, and Kemp's ridley presented 22 individuals. However, the species presented the most entanglement was the loggerhead (*C. caretta*).

Figure 7. The color of plastic that were found in the gastrointestinal system from sea turtles found on the systematic review. The following colors can be seen in the color classification scheme: red, yellow, blue, orange, pink, grey.

Figure 7 shows what color of plastic sea turtles are most susceptible to eat. Only in 10 out of the 42 papers found in the systematic review presented a color classification of plastic the sea turtle had ingested plastic. In some papers, white and transparent are considered a different category or classification, while in others they are considered the same color classification. White or transparent plastic is the most common color of plastic ingested, which can be misplaced as their food or can instead be attached or tangled with actual food resources.

Threat Score by species

Table 2. Scoring criteria for the species distribution, IUCN Red List status, entanglement literature and ingestion literature categories.

Table 3. Scores for the seven species of sea turtles.

Table 4. Table of the seven species of sea turtles with they're the score from Table 3. Endemism status, conservation status, and unreferenced population were determined by the IUCN Red List Status (https://www.iucnredlist.org/).

Figure 8. Summary of priority scoring analysis for the seven species of sea turtles found across the globe and plastic contamination. Scoring elements include species distribution, IUCN Red List status, and evidence from literature for harm from plastic contamination caused by ingestion. Each element was scored (0-3) and combined to give a final priority score, distributed through the final column. In the section of evidence from literature, NE (E), Mod (E), and Maj (E) represent the evidence from the literature to entanglement; however, NE(I), Mod (I), and Maj (I) represent the evidence from literature of plastic ingestion. The (E) represent entanglement and (I) represent plastic ingestion. In the vertical axis, the total number of individuals found and in the horizontal are the categories given for distribution, IUCN Red List Status, Evidence from literature (entanglement and plastic ingestion), and the threaten score.

In Table 3, it shows the valued each species of sea turtle got to calculate the threat score. In Table 4, it shows the distribution, the population around the world and examples from evidence for entanglement and plastic ingestion. In Figure 8, values of 0-3 were given for each score, with 0 or 1 being the lowest and 3 being the highest. In species distribution, only two species were given the number 2, which means they are native. In terms of the evidence literature score, all the species were given the highest score, because in the 42 papers found, the species are mentioned at least one time. The species that received the highest score was Kemp's Ridley (*L. kempii*) with $I = 18$ and $E = 12$. However, the species that reported the most plastic ingestion was the green turtle (*C. mydas*).

The smaller the *p*-value, the stronger the evidence for rejecting the null hypothesis. This leads to the guidelines of *p* < 0.001 indicating very strong evidence against H0, *p* < 0.01 strong evidence, $p < 0.05$ moderate evidence, $p < 0.1$ weak evidence or a trend, and $p \ge 0.1$ indicating insufficient evidence. Two paired T-tests were made to identify any significant differences between the evidence of plastic ingestion versus evidence from entanglement; and the other one was made between the threaten score from plastic ingestion. The first t-test generated the following results: the *p* value was 0.1723 and the significance for *p* value was ≤ 0.01 ; indicating no significant differences between evidence of entanglement and plastic ingestion. The same result was obtained with the threat score of plastic ingestion and entanglement. The *p* value was 0.1996 and the significance for the *p* value was < 0.01; indicating no significant difference between the threat score of plastic ingestion and entanglement.

DISCUSSION

Sea turtles and plastic ingestion

Since plastic ingestion has been reported in the seven species of sea turtle, the quantity and type of plastic ingested changes due to differences in habitats and feeding preferences (Digka et al. 2020). Immediately after plastics are ingested by sea turtles, either actively (by mistaken plastic items for pray), indirectly (feeding on animals which previously ingested plastic or in other words, via bioaccumulation), or accidentally, they mostly accumulate within the gastrointestinal tract due to the inability of the animal to regurgitate these items (Biagi et al. 2021). Ingested plastic may cause death by blockage, perforation of the digestive tract or sublethal effects like dietary dilution or exposure to chemicals (Bjorndal et al. 1994, Jerdy et al. 2017, Vélez-Rubio et al. 2018). Death due to involvement with fishing activity isthe greatest anthropogenic source of mortality of sea turtles affecting mostly juveniles and sub-adults. In terms of plastic identification, there are currently methods being done in both dead and living individuals.

Methods for plastic identification and extraction

Dead turtles.

Many studies have researched the presence of plastic pollution in all the species of sea turtle, but most of them were performed on gastrointestinal tracts taken from dead animals. Small amounts of ingested plastic could be dangerous to their health and causing their death (Bugoni et al. 2001, da Silva Mendes et al. 2015). In some Brazilian cities, there is black market for whole turtle shells for decorative purposes (Bugoni et al. 2001). As shown in Figure 3, seven studies were done in Brazil (Bugoni et al. 2001, da Silva Mendes et al. 2015, de Carvalho et al. 2015, Jerdy et al. 2017, Rizzi et al. 2019, Miguel et al. 2020, Petry et al. 2021).

For the methods used to identify plastic ingestion on dead sea turtles, a necropsy was made to determine the cause of death of the individuals (Lazar and Gračan 2011, Matiddi et al. 2017, Domènech et al. 2019, Eastman et al. 2020). The Curve Carapace Length (CCL) was obtained from every individual in order to identify their age and whether they were posthatchling, juvenile, or adult individuals (Bugoni et al. 2001, Hoarau et al. 2014, Yaghmour et al. 2018, Rice et al. 2021). The gastrointestinal tract was removed from the body cavity (Bjorndal et al. 1994) and separated into the esophagus, stomach, and intestines (small and large). Each section was weighed to obtain the wet mass and individually weighed (Digka et al. 2020). The digestive tract was frozen at -20ºC for analysis in the labs for plastic identification and preserved in 70% ethanol (Yaghmour et al. 2018, Eastman et al. 2020, Rice et al. 2021). Every section was emptied and washed on top of three stacked metallic sieves of different mesh size (5 mm, 1 mm, and 300 μm). Each sieve was washed completely and examined for plastic. Plastics were classified, counted, and weighted (Digka et al. 2020). Additionally, in one study, they performed a metal identification analyzed in the liver, muscle and kidney of the sampled turtles (Sinaei et al. 2021).

Alive turtles.

For the living turtles, plastic identification used alternate methods that involved extracting the gastrointestinal system (esophagus, stomach, small and large intestine). Two different methods to identify if the individuals has ingested plastic were used by different authors (Franzen-Klein et al. 2020, Biagi et al. 2021).

The first method was used on rescued loggerhead sea turtles (*C. caretta*) that were found stranded or captured by fishing nets. The turtles were held at the Sea Turtle Rescue Center to cure and rehabilitate them, in which they were hosted in single fiberglass tanks or tanks that were separated by a septum and were fed twice a week with fishery products until release. All the turtles were given a name, measured (Curved Carapace Length, CCL), and included the days they were hospitalized as well as the sampling date. Feces produced by the sea turtles at the rescue center was collected from the tanks with a metallic net that was washed twice every time using ultrapure Milli-Q water and was placed into sterilized glass containers. Feces must be collected as soon as possible after production in order to avoid having long periods of time in contact with water, mainly for the fact that the animals share a tank. The samples were frozen at -20ºC and were transported to the laboratory using coolers with ice packs. Samples were stored at – 80ºC until analysis could be made. A subsample of 0.2 g of fecal matter was weighted and placed in a glass beaker with 40 mL of 10% of KOH for the degradation of the organic sample. Each glass beaker was covered with a glass cap. Samples were incubated overnight at 40ºC with uninterrupted stirring. To reduce external contamination, the work was done under a fume hood, all surfaces were wiped with alcohol, and each investigator used a lab coat and rubber gloves. All the equipment that was used during the analysis was rinsed with 10% HCl solution. While all the precautions were taken in order to prevent contaminations, a control was made containing only 40 mL of 10% KOH with all the samples. Samples were pre-filtered using a 1 mm sieve and afterward filtered under a vacuum filtration system with a 1.2 μm pore size. The filtration system was closed with a glass dish to prevent contamination. Filters were placed in closed double glass dishes and covered with aluminum to protect from light, which could lead to fragmentation of the polymers in the samples. Every filter was left to dry for one day at room temperature under the fume hood. Negative controls were compared with the samples, allowing for detection of possible contamination with every set of analysis (Biagi et al. 2021).

The second method was used on 1 loggerhead (*C. caretta*), 1 Kemp's Ridley (*L. kempii*), and 4 green turtles (*C. mydas*) in which liquid contrast, barium-impregnated polyethylene spheres (BIPs), ultrasonography, endoscopy, and computed tomography (CT) was used to diagnose marine debris-induced foreign body obstructions in 3 species of sea turtle (Franzen-Klein et al. 2020).

While each method is highly effective in identifying plastic in sea turtles, the first method is more practical because it helps identify how many items of plastic were found in each filter, how many items of plastic were found digested in the organic matter, quantifies the amount found in the feces of the turtles, and allows the turtle to be kept at a rescue center and be monitored regarding the effects of plastic ingestion. If fishing hooks have been ingested, they can be detected with a radiograph; if there are lines that are retained in the digestive tract, their presence can be assessed only through necropsy (Casale et al. 2010).

Sea turtles and entanglement

Entanglement in marine debris is the most visible effect of plastic pollution on marine organisms and has been reported to affect more than 344 marine species (Digka et al. 2020). Entanglement in fishing nets is the main cause of death of sea turtles (Sinaei et al. 2021) and has been reported at all stages (Duncan et al. 2017). Hooked turtles are not hauled aboard as fishermen often cut the lines as soon as they identify where the hook is embedded (Blasi and Mattei 2017). If the hook line is ingested, they might produce intussusception and other gut pathologies that can be lethal to the turtle (Blasi and Mattei 2017). Entanglement in plastic debris or ghost-fishing gear of fish aggregating devices represented the primary cause of problems, but also represented a secondary source of injury to sea turtles (Blasi and Mattei 2017). Corticosterone, blood levels, and plasma biochemistry are made to determined how much stress the turtles were experienced while entangled (Wilcox et al. 2013, Jensen et al. 2013, Hunt et al. 2016, Miguel et al. 2020).

CONCLUSION

After analyzing the 42 articles included in this review, the main and specific objectives of this study were found to be fulfilled. There is evidence in studies reporting plastic ingestion since 1987 and in one study, they collected the data from 1885 to 2007 (Mrosovsky et al. 2009). Entanglement and plastic ingestion by sea turtles has become a severe problem for the seven species found around the world. As marine plastic pollution increases and persists in the ecosystem, understanding its effects on sea turtle populations is a high priority. New studies on sea turtles must be done to quantify how the seven species are being affected at a global level. Most of the turtles are currently considered vulnerable or endangered by the IUCN and marine plastic pollution is causing reduction in their populations.

One limitation of this review was the relative low number of studies included. For future studies, it is recommended to use more databases to obtain more results, expand searches in Google Scholar, and increase the year range to have more papers and more results. Two global studies were found, one with plastic ingestion (Schuyler et al. 2014) and the other with entanglement (Duncan et al. 2017). Another recommendation would be to do a metanalysis in order to refine the statistical analysis and further test data. It is recommended for future systematic reviews or metanalyses that collect data to validate their results using statistics.

Finally, the new method to identify plastic ingestion by sea turtles must be done around to see how affected sea turtles' population regarding plastic ingestion. This new method allows for plastic identification and analysis in living sea turtles. A group of marine biologists (including me) and veterinarians got the opportunity to work with this method. This is a method that can prove the ingestion of microplastics on sea turtles found on Machalilla/ Isla de la Plata and San Cristobal Island. Additionally, this review serves to evaluate how affected sea turtles have interacted with plastic, from entanglement with marine debris to plastic ingestion, and what methods can be done to identify the plastic that has been ingested. Most importantly this study serves as a call to action to protect sea turtles. The change starts with you.

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APPENDIX

Appendix A. Table of search terms.

Appendix B. Combination of the search terms used in the two databases.

Appendix C. Table of summary of the literature research.

