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A best practice framework for assessing plastic ingestion in marine turtles

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ABSTRACT

The ingestion of plastic debris has been reported in all seven marine turtle species, affecting vital processes throughout their entire life cycle and key habitats. Consequently, this emerging threat has been recognized as a priority conservation concern. The potential health impacts range from cryptic sublethal effects to severe injury and death. A comprehensive understanding of these impacts and the processes involved, at both the individual and population levels, is crucial for evaluating the vulnerability of marine turtles to plastic pollution. Aiming to guide researchers and stakeholders from the initial stages of project development, this study discusses essential components for establishing and achieving research on plastic ingestion in marine turtles. Drawing on diverse efforts globally, this manuscript compiles the most common approaches and established methodologies, while evaluating resource availability and capabilities, to outline a globally applicable best practice framework for designing and implementing research and monitoring initiatives on plastic ingestion impacts to marine turtles.

1. Introduction

The ingestion of plastic debris has been reported in all seven marine turtle species (Duncan et al., 2019b; Lynch, 2018; Schuyler et al., 2014a), affecting vital processes across their entire life cycle and key habitats (Do Sul et al., 2011; Duncan et al., 2021; Schuyler et al., 2014a). Plastic ingestion, therefore, has been recognized as an emerging threat and a priority conservation concern for marine turtles (Fuentes et al., 2023; Hamann et al., 2010; Nelms et al., 2016; Senko et al., 2020).

Marine turtles are believed to be particularly vulnerable to the impacts of plastic ingestion due to their long-life spans, complex life history and migratory behavior (Duncan et al., 2021; Lynch, 2018; Santos et al., 2015). The impacts caused by the ingestion of plastic on the health of marine turtles are diverse, ranging from negligible to lethal. The severity of physical impacts is primarily determined by the quantity and characteristics of the ingested plastics (Duncan et al., 2019a; González-Paredes, 2024; Rizzi et al., 2019; Santos et al., 2015). Large pieces of plastics or significant quantities can cause the blockage of the digestive tract (Rizzi et al., 2019; Vélez-Rubio et al., 2018), which can eventually result in ischemic necrosis and septicaemia with lethal consequences (Mashkour et al., 2020; Tagliolatto et al., 2020). Among the severe impacts reported are also abrasions and lacerations of the digestive tract caused by sharp or pointed plastics (Camedda et al., 2014; Derraik, 2002; Lazar and Gračan, 2011). Furthermore, the displacement of dietary items by ingested plastic can reduce stomach capacity and affect the stimulus to feed, leading to dietary dilution and malnutrition (McCauley and Bjorndal, 1999; Santos et al., 2020; Tourinho et al., 2010). On the other hand, the potential bioaccumulation of toxic substances leached from ingested plastic into blood and tissues may cause cryptic sub-lethal effects. The absorption of plasticizers may lead to malfunctions in metabolic and endocrine systems, as well as disorders in somatic growth rates and reproduction (Clukey et al., 2018; Nelms et al., 2016; Savoca et al., 2023).

Understanding the full extent of these impacts, and the mechanisms involved, is crucial to assessing the vulnerability of marine turtles to plastic pollution. In recent years, reports on plastic ingestion in marine turtles have increased, transitioning from largely opportunistic observations to more systematic and structured studies. Consistent data collection has been crucial in understanding the scope and trends of plastic ingestion in marine turtles, particularly through studies based on long-term stranding networks (Choi et al., 2021; Domènech et al., 2019) and those relying on large-scale bycatch monitoring programs (Clukey

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et al., 2018; Fukuoka et al., 2016). Additionally, significant regional efforts have been made to establish guidelines for monitoring the impact of plastic pollution on marine megafauna, such as the MSFD (Marine Strategy Framework Directive) and the OSPAR Convention (Convention for the Protection of the Marine Environment of the North-East Atlantic) in Europe, which protocols have been adapted for marine turtles by Galgani et al. (2013). Furthermore, the INDICIT Consortium employs loggerhead turtles (*Caretta caretta*) as bioindicators to assess plastic pollution levels in the Mediterranean basin, in alignment with the objectives of the Barcelona Convention (Darmon et al., 2022; Fossi et al., 2018; Matiddi et al., 2019). Similarly, in the United States, the project BEMAST (Biological and Environmental Monitoring and Archival of Sea Turtle Tissues), a long-term biobanking initiative, archives marine turtle blood and tissue samples for real-time and retrospective contaminant analysis related to plastic pollution (Savoca et al., 2022; Shaw et al., 2021). At a global scale, international initiatives like the Global Plastic Ingestion Bioindicators (GPIB) promote the use of marine turtles as bioindicators to generate critical insights into the trends, risks, and impacts of plastic pollution on species and ecosystems (Savoca et al., 2024a).

The consistent application of standardized procedures and protocols, combined with the sharing of data and results in open-access repositories, ensures data comparability, which is crucial for advancing our understanding of plastic ingestion in marine turtles and enabling a

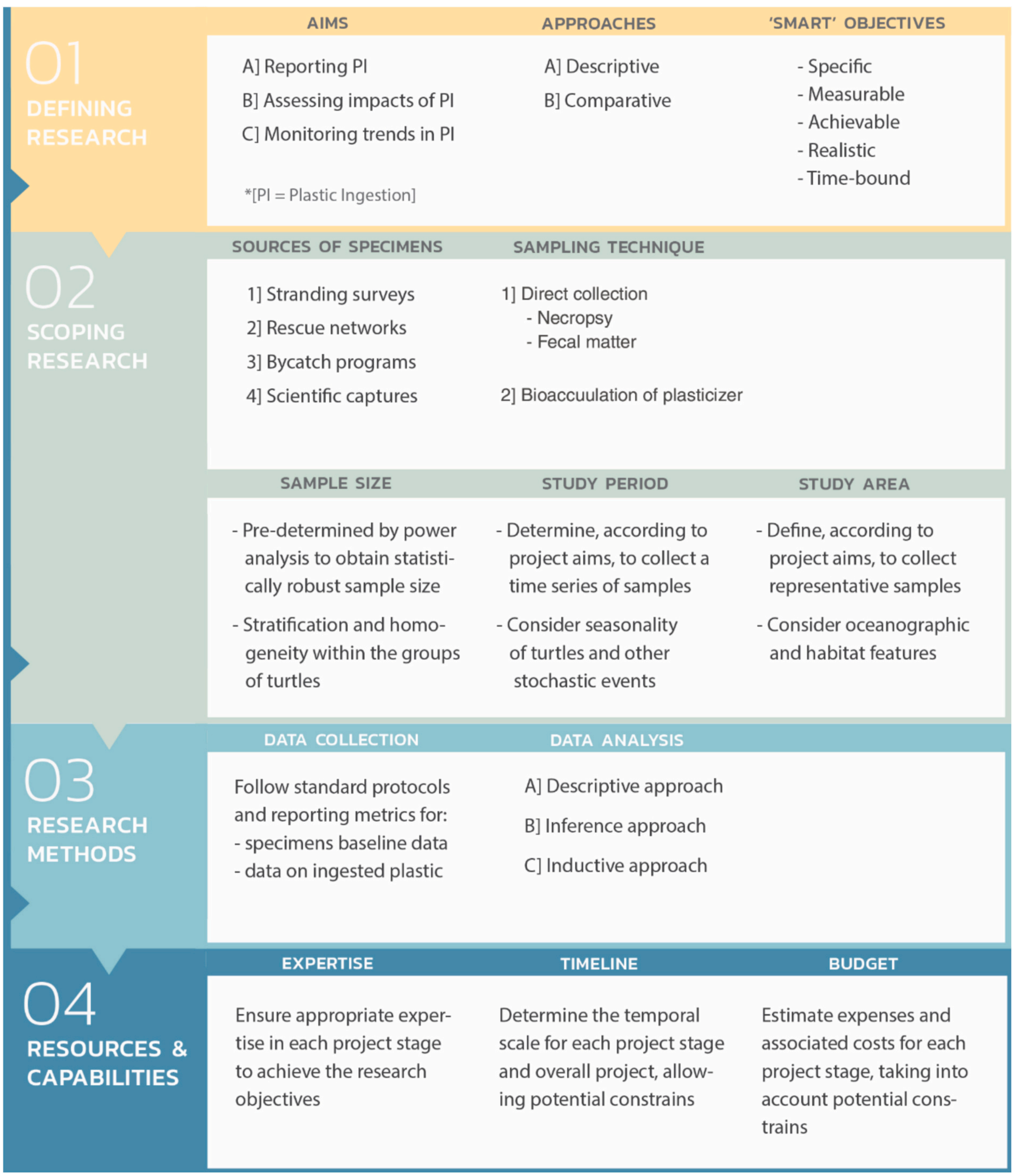

Fig. 1. Conceptual map of the proposed best practice framework for assessing the impact of plastic ingestion in marine turtles.

broader impact assessment of plastic pollution at both the population and species levels (Fuentes et al., 2023; Hamann et al., 2010; Nelms et al., 2016; Senko et al., 2020). Furthermore, building on the need for consistency, a coherent project design aligned with available resources and capabilities is critical for ensuring research feasibility, meeting objectives, and addressing constraints like funding, time, and personnel. In addition, selecting appropriate methodologies, optimizes data collection, identifies gaps, and allows for timely adjustments to be made.

Here we compile the most common approaches and established methodologies to outline a globally applicable best practice framework for researching plastic ingestion in marine turtles. The document has been informed by the literature, alongside the collective experience of collaborators and discussions among experts, incorporating insights gained through practical research and fieldwork. It outlines key components for setting research objectives, standard methodologies, and strategies to enhance monitoring efforts, aiming to guide researchers and stakeholders in effectively assessing plastic ingestion.

2. A best practice framework

Achievement of research goals largely depends on establishing a well-designed research plan before project commencement. In this context, a framework represents a conceptual structure for the theoretical and technical background essential to designing efficient research plans by articulating strategies based on common research methods and replicable techniques. Below, key aspects of a best practice framework are discussed, including setting clear research objectives and defining the scope, selecting appropriate approaches and methods for data collection and analysis as well as understanding the biases associated with these approaches, and evaluating resources and capabilities (Fig. 1).

2.1. Define research aims, objectives and approaches

There are a wide diversity of research aims concerning plastic ingestion in marine turtles. These can be grouped into three main categories according to their primary objectives (these three categories will serve as reference points throughout this document):

A] Reporting plastic ingestion.

This refers to those projects reporting the ingestion of plastic debris by marine turtles, ranging from opportunistic to more extensive and systematic studies. Along with quantities and characteristics of ingested plastic, parameters such as frequency of occurrence, incidence, rates and patterns of plastic ingestion are reported, provided a representative sample size can be obtained (Duncan et al., 2019a; Galgani et al., 2023; Gama et al., 2021; González-Paredes, 2024; Vélez-Rubio et al., 2018).

B] Assessing impacts of plastic ingestion on the health of turtles.

These projects aim to assess the impact caused by plastic ingestion at individual or group levels, evaluating, when possible, the factors involved in the threatening process. These studies may span multiple disciplines, including toxicology, analytical chemistry, and veterinary diagnostic assessments (Sala et al., 2021; Savoca et al., 2018).

C] Monitoring plastic ingestion over time.

Projects built upon the aims [A] or [B] can serve as a basis for monitoring purposes, which involves assessing a subset of animals over time by establishing longer-term objectives and adhering to common, well-established methods. Such studies enable an evaluation of the incidence of plastic ingestion and the elaboration of trends across different temporal and spatial scales (Choi et al., 2021; Darmon et al., 2022; Domènech et al., 2019).

Establishing clear, unambiguous research objectives must underpin project aim(s). SMART objectives (Specific, Measurable, Achievable, Realistic and Time-bound) enable a focus on the specific and achievable research question(s), which can be addressed using a specific approach (es) to attain quantitatively and/or qualitatively measurable data in a defined timeframe (Doran, 1981). The objective(s) must be realistic and feasible, hence the need to assess potential limitations and biases and evaluate the availability of resources and capabilities (concepts developed in the sections below). It is equally important to ensure that results are statistically robust. Comprehensive data collection, combined with a large and stratified sample size, allows reliability and representativeness in the inferences drawn from analyses.

Research and monitoring plastic ingestion in marine turtles can be approached from a *descriptive* or *comparative* perspective. Descriptive reports range from opportunistic findings associated with studies where assessing plastic ingestion is not the primary goal (e.g., bycatch monitoring programs or stranding networks collecting and examining dead turtles, in which plastic ingestion is detected after routine necropsy examinations; see Da Silva Mendes et al., 2015); to projects with a higher degree of planning and research complexity for assessing this threat, involving a representative subset of animals to enable meaningful results and/or infer cause-and-effect relationships (e.g., evaluation of trends in plastic ingestion by green turtles in the Gulf of Mexico for three decades; see Choi et al., 2021). Comparative analyses use systematic methods to understand the general principles of plastic ingestion by identifying differences and similarities among distinct groups of study. These could also apply to analysis based on understanding variation or interpretation of diversity to establish statistical relationships between two or more datasets (e.g., analysis of plastic ingestion patterns in different marine turtle species caught as bycatch in pelagic longline fisheries; see Clukey et al., 2018).

2.2. Scoping the research

The scope of the research or monitoring project describes the extent to which the field of study will be explored. It defines the parameters within which the study will be developed, including source of samples, sampling frequency, sample size, extent of the study area, project duration, types of data and subsequent analysis.

2.2.1. Source of specimens

The sources of specimens for research on plastic ingestion in marine turtles can be grouped into two main categories, *dead* or *live* turtles. The primary sources of dead specimens include (1) stranding and rescue networks, where turtles are collected stranded dead on the coastline or die after unsuccessful recovery from rescue attempts, and (2) bycatch programs systematically retrieving dead turtles from active fishing gear. Among the major sources of live specimens are (3) rescue networks and bycatch programs collecting injured turtles, and (4) *in-water* projects capturing turtles into the wild for monitoring and research purposes.

Dead turtles opportunistically collected might have been in poor health conditions before encountering, exhibiting abnormal feeding behaviors and/or habitat use. This has the potential to lead to either an underestimation or overestimation of plastic ingestion rates in comparison with the overall rate of a stock or population (Lynch, 2018; Casale et al., 2016; González-Paredes, 2024). Nevertheless, these turtles can provide valuable insights into the impact caused by plastic ingestion at the individual level (González-Paredes, 2024). In contrast, turtles collected systematically are considered more reliable sources of specimens, as they likely better reflect the overall exposure to plastic ingestion of a stock or population (Casale et al., 2016; González-Paredes, 2024) (Table 1).

The foraging ecology of the study species should be carefully considered since the likelihood of plastic ingestion is closely linked to interspecific feeding strategies (Lynch, 2018; Schuyler et al., 2014a). Opportunistic foraging species such as the loggerhead turtle (*Caretta*

Table 1
Strengths and limitations of the multiple approaches included in the proposed best practice framework for assessing the impact of plastic ingestion in marine turtles.

Approach	Strengths	Limitations
Specimen source		
• Bycaught and wild-capture turtles	Indicators of the overall population's exposure to plastic ingestion	Sampling must be systematic
• Stranded and rescued turtles	Provide valuable insights into the severity of the impact of plastic ingestion	Potential biases in plastic ingestion rates due to pre-existing health issues, leading to abnormal feeding behavior and/or habitat use
Sampling method		
• Necropsy	Allow to retrieve all the digestive contents for assessing plastic ingestion	Only applicable to dead turtles, potential biases according to the specimen source
• Fecal matter monitoring	Allow to assess plastic ingestion in live turtles	The minimum monitoring period needs to be adjusted to the upper limit of gastrointestinal transit time No data is provided regarding the location of plastics along the digestive tract
• Gastric lavage	Allow to assess plastic ingestion in live turtles	Only a small portion of the digestive content can be retrieved from the oesophagus and stomach Non-efficient method for assessing plastic ingestion
• Analysis of plasticizer bioaccumulation	Allow to assess sub-lethal effects associated with the toxics leaching of plastic into blood or tissues	Harmful levels of plasticizer accumulation remain unclear Need to differentiate from the assimilation of chemicals from background ocean pollution

caretta) are potentially exposed to consuming a wider variety of plastics because of their low discrimination in selecting dietary items (Lynch, 2018; Schuyler et al., 2014a). While specialist feeders such as the leatherback turtle (*Dermochelys coriacea*), feeding mainly on gelatinous organisms, are more likely to ingest soft plastics resembling their prey (Constantino and Salmon, 2003; Mrosovsky et al., 2009; Schuyler et al., 2014b). Furthermore, feeding strategies are subject to adaptive changes across life stages or according to food accessibility; as in the case of juvenile green turtles (*Chelonia mydas*) at the oceanic stage, which exhibit opportunistic feeding behavior, making them potentially more exposed to the risk of plastic ingestion (Gama et al., 2021; González-Paredes, 2024; Vélez-Rubio et al., 2016). Additionally, early life stages are potentially more vulnerable to internal injuries because of their narrow digestive tract relative to the size of plastic particles (Boyle, 2006; Schuyler et al., 2012). Other authors suggest that the longer digestive tracts of adults and sub-adults could retain greater amounts of plastic debris for longer than small animals (Casale et al., 2016; Wilcox et al., 2018). While species and age/size class can serve as predictors of plastic ingestion, specific individual-level differences may occur in relation to habitat use, feeding behavior, and diet (Duncan et al., 2019b, 2021; Casale et al., 2016; Lynch, 2018; Nelms et al., 2016; Schuyler et al., 2014b).

2.2.2. Sampling technique

Assessing plastic ingestion in marine turtles can be approached through two primary methods: (1) direct collection and analysis of ingested plastics, or (2) analysis of plasticizer bioaccumulation within the organism (Table 1).

1] Direct collection of ingested plastic.

The ingested plastic can be collected from gastrointestinal contents retrieved through necropsies of dead animals, by examining fecal matter, or from material obtained via gastric lavage in live animals (Casale et al., 2016; Nelms et al., 2016).

Necropsy allows for the extraction of the entire digestive contents (see methods in Wyneken, 2001) and the examination of all digestive tract sections to determine the presence and distribution of ingested plastics (see methods in Matiddi et al., 2017; Duncan et al., 2021). This technique remains the most reliable procedure for analyzing plastic ingestion by an individual.

Fecal matter examination facilitates the assessment of plastic ingestion in live animals while concurrently evaluating health status. This method is typically applied to turtles in rehabilitation facilities or those captured in the wild and temporarily held in captivity for research purposes (see methods in Casale et al., 2016; González-Paredes et al., 2021; Fukuoka et al., 2016; Hoarau et al., 2014). In such cases, the monitoring period required must be longer than the upper limit of the ingesta passage time to maximize the likelihood of collecting from the feces all potential plastic previously ingested in the environment (González-Paredes et al., 2021; Valente et al., 2008). The monitoring period must be individually tailored and extended until the animal has fully recovered, and no plastic is detected in its feces (González-Paredes, 2024). Additionally, this methodology could also serve as an early warning of digestive disorders or obstructions caused by plastic ingestion (González-Paredes et al., 2021).

Although gastric lavage can also be used for examining digestive contents in live turtles (see methods in Forbes and Limpus, 1993; Stokes et al., 2008), this technique is not recommended, as it only allows for the collection of an unknown proportion of the esophageal and stomach contents, preventing quantification of plastics remaining in the intestines, where most accumulation has been observed (Camedda et al., 2014; Duncan et al., 2019a). Furthermore, this technique involves a risk of internal lacerations and perforations of the digestive tract if not conducted carefully by specialists with appropriate training and expertise (Manire et al., 2017).

The direct collection of ingested plastic is considered the most recommended method for assessing the physical impacts caused by plastic ingestion (Casale et al., 2016; Lynch, 2018; Nelms et al., 2016). Plastic retrieved through any of these techniques can be considered representative of the plastic consumed by the examined turtle. However, it should be taken into consideration potential variations in retention times associated with particular types of plastic or their dimensions as well as influences of the turtle's health status on the progression rates of plastic along the digestive tract.

2] Analysis of plasticizer bioaccumulation within the organism.

The polymer bonds of plastics are susceptible to breakage during digestion, facilitating the lixiviation of toxic substances, commonly known as plasticizers, which can eventually be absorbed into blood and tissues (Sala et al., 2021; Savoca et al., 2023). Chronic exposure to plasticizers, due to the prolonged digestive process in marine turtles, may lead to sub-lethal effects, including metabolic and endocrine disruption, alterations in growth rates, or impaired fertility (Clukey et al., 2018; Nelms et al., 2016; Rowdhwal and Chen, 2018; Savoca et al., 2023). In addition, it has been reported that these toxins can be transferred from the nesting female to their eggs during the ovarian maturation (De Andrés et al., 2016; Savoca et al., 2024b).

Among the plasticizers most studied in marine turtle toxicology are the organophosphate esters (OPEs) and phthalate esters (PAEs) (Table 1). Analysis to determine the accumulation levels of OPEs typically involves muscle tissue (see methods in Sala et al., 2021); nonetheless, it can also be performed using plasma samples from live turtles (see methods in Solé et al., 2022; Omedes et al., 2024). Similarly, PAEs can be detected in both dead turtles, sampling gonads and liver, and live turtles, through biopsy of fat tissues (see methods in SanJuan et al., 2023; Savoca et al., 2018).

Knowing the accumulation levels of these plasticizers in the organism can enable the assessment of cryptic sub-lethal effects caused by plastic ingestion. Nevertheless, it should be considered that the leaching of plasticizers can also occur in the environment due to photochemical and mechanical forces fragmenting and degrading plastic (Andrady, 2015; Kershaw and Rochman, 2015). Therefore, it is central for these analyses to discern between the bioaccumulation of toxic substances from ingested plastics and their assimilation from background ocean pollution (Koelmans et al., 2021; Savoca et al., 2023).

2.2.3. Sample size

The sample size should be pre-determined according to the source of specimens, the sampling technique and intended analyses. In the early stages of the project design, it is recommended that power analyses are used to estimate the minimum sample size required for statistically robust analysis and to detect varying levels of difference (see methods in Lavers et al., 2021; Provencher et al., 2015). In addition, post-hoc analysis can validate the data collection and approach and set the monitoring framework for ongoing research (Gillett, 1994; Lavers et al., 2021).

Reports of plastic ingestion are generally less focused on obtaining pre-determined sample sizes, particularly when data are collected opportunistically. The key component in these studies is prioritizing methodological consistency through standard protocols for sample collection and analysis, ensuring data comparability across diverse sources. Evaluating potential biases from non-representative animals, such as sick individuals or those with abnormal behaviors, is equally important. Homogenizing study groups (e.g., age classes, habitat uses, dead vs. live turtles, etc.) helps mitigate these biases, enhancing the reliability of analyses and the interpretation of results.

For assessing impacts of plastic ingestion, large and well-stratified datasets are crucial for ensuring reliable results and drawing meaningful conclusions regarding the factors influencing the process. This is particularly important, as establishing cause-effect relationships between ingestion rates and health impacts remains challenging without controlled dose-response trials, which are restricted due to ethical considerations regarding marine turtles.

Monitoring projects require systematic sample collection using standard methods across time to generate datasets with sufficient statistical power, enabling the inference of plastic ingestion incidence and trends within a group of animals or a stock population.

2.2.4. Study period

The presence and aggregation of turtles may vary across time, even at small temporal scales, due to breeding and nesting seasons and migratory patterns among other stochastic events (Meylan et al., 2011; Schuyler et al., 2014a). Furthermore, the occurrence and abundance of plastic are also subject to variations at temporal and spatial scales due to abiotic factors (wind patterns, ocean currents, coastal fronts, river discharge) or episodic and stochastic events (heavy rainfalls, cyclones, natural or anthropogenic disasters) (Cózar et al., 2014; Kershaw and Rochman, 2015).

The study period required for reports of plastic ingestion varies depending on the research aims (see Section 2.1), and whether data collection is conducted opportunistically (reporting isolated incidents) or systematically (referring to extended periods). In assessments of plastic ingestion impacts, the study period is determined by the methodology employed and sample size; necropsies are conducted on each individual as needed, while fecal examination requires extended monitoring periods, tailored individually to each case (see Section 2.2.2). The length of the study period is particularly relevant when developing monitoring programs, which require sufficient duration to collect a time series of representative sample sizes to assess incidence and trends in plastic ingestion over time and space.

2.2.5. Study area

The distribution and concentration of plastics are not uniform in the environment. Hence, the study area and its defining biophysical features should be assessed and considered as potential predictors of plastic ingestion. Plastics are often concentrated in oceanic gyres and coastal fronts by the combined actions of winds and currents. Land-based waste sources, discharging rivers and frontal systems also generate aggregation zones of debris. As a result, the risk of plastic ingestion increases significantly when these areas with high loads of plastic overlap with habitats occupied by marine turtles (González-Carman et al., 2014; Schuyler et al., 2016). Furthermore, behaviors guiding early life stage turtles to specific areas with high levels of plastic pollution for feeding and development could pose an evolutionary trap for the species (Duncan et al., 2021; Santos et al., 2021).

The study area in reports of plastic ingestion may either refer to locations where cases were opportunistically recorded or extend to a broader area where data are being collected systematically. This similarly applies to impact assessments and monitoring programs; however, it is recommended that study areas are expanded to ecologically relevant scales that could use to infer the incidence and trends of plastic ingestion at stocks or a population level (e.g., zones of debris aggregations, pelagic feeding areas, specific foraging grounds).

2.3. Research methods

Research methods are devised to provide appropriate techniques and tools for sample and data collection, and procedures for subsequent analysis and interpretation of results. Standard protocols and reporting metrics enable robust statistical analysis, repeatability, and the comparison of results. Before deciding on the most suitable method for achieving the research goals, exploring and considering the scope, purpose, and applicability of the available techniques is essential.

2.3.1. Data collection

2.3.1.1. Baseline data of study animals/turtles examined. Essential information on study individuals (e.g., species, sex, age class, size, weight), should be gathered alongside information on their health status (e.g., physical condition, health assessment, injuries, cause of death). These data should be gathered using common fieldwork protocols, standard veterinary procedures and/or established necropsy examinations (see methods in Eckert et al., 1999; Matiddi et al., 2019; Rodríguez-Baron et al., 2016; Wyneken et al., 2013).

Assessing impacts or inferring trends also requires the collection of additional data on parameters acting as drivers of plastic ingestion. These include biological factors (e.g., feeding behavior, habitat use, migratory movements, occurrence seasonality), as well as oceanographic features (e.g., neritic zone, pelagic environment, debris aggregations, currents).

Additional methods for data collection may apply depending on specific research objectives. For example, studies using satellite telemetry data to analyze overlaps of turtle habitats with aggregation zones of plastic debris (see González-Carman et al., 2014); or experimental methods to evaluate responses of turtles to airborne odorants emanating from biofouled plastic (see Pfaller et al., 2020).

2.3.1.2. Baseline data describing ingested plastic. Determining the size range of plastics to be analyzed is essential, as it defines the appropriate

Table 2
Standard protocols and reporting metrics for the quantification, classification, and characterization of ingested plastic.

Analysis	Reporting metric	Objective	References
Quantification	Units of plastic pieces	Number of plastic pieces retrieved (total or per digestive tract section) ingested by a single turtle or a sampled group.	Hoarau et al. (2014); Rice et al. (2021); Wilcox et al. (2018); Yaghmour et al. (2018)
Occurrence	Frequency of Occurrence (%FO)	Proportion of sampled turtles presenting plastic ingestion or percentage of a plastic category over the entire sample.	Choi et al. (2021); Domènech et al. (2019); Matiddi et al. (2017); Rizzi et al. (2019)
Categorisation	Plastic category	Classification of ingested plastic based on their typology.	Darmon et al. (2022); Galgani et al. (2013); Matiddi et al. (2019); Rodríguez et al. (2022); Solomando et al. (2022)
Dry weight	Grams of ingested plastic	Mass of ingested plastic (total, per plastic category or per piece) by a single turtle or sampled group.	Camedda et al. (2014); Colferai et al. (2017); Nunes et al. (2021); Pham et al. (2017); Schuyler et al. (2012)
Body burden	Grams of ingested plastic/Kilograms of turtle weight	Relation between mass of ingested plastic and turtle weight.	Clukey et al. (2017); Domènech et al. (2019); Duncan et al. (2021); White et al. (2018)
Volume	Cubic millimetres (3D)	Volume of plastic ingested (total, per plastic category or per piece) by a single turtle or a sampled group.	Clukey et al. (2017); Domènech et al. (2019); Godoy and Stockin (2018); González-Paredes, (2024); Vélez-Rubio et al. (2018)
Colour	Colour category	Colour of plastic pieces retrieved based on standard charts, including the visible spectrum, black, white and clear/transparent.	Duncan et al. (2019a); Eastman et al. (2020); Fukuoka et al. (2016); Santos et al. (2015); Schuyler et al. (2012)
Sharpness and Flexibility	Sharpness Index and flexibility Index, based on a three value-scale for each characteristic	Shape and stiffness of each plastic particle retrieved as index of impact severity.	González-Paredes, (2024); Rizzi et al. (2019); Schuyler et al. (2014b); Yaghmour et al. (2021)
Buoyancy	Positive (floats at surface), negative (sinks), or neutral (floating in the water column).	Buoyancy of plastic particles retrieved as an indicator of where in the water column the plastic was ingested (surface, bottom or in the water column).	Fazey and Ryan (2016); Reisser et al. (2015); Rumbold et al. (2020); Vélez-Rubio et al. (2018)
Polymer composition	Polymer composition	Characterization of the polymer composition of the plastic particles retrieved through FT-IR or Raman spectrophotometry analysis.	Bruno et al. (2022); Camedda et al. (2022); Digka et al. (2020); Jung et al. (2018); Prampramote et al. (2022); Rice et al. (2021); Rizzi et al. (2019)

methods for their extraction and isolation. Commonly used size boundaries are macro- (>25 mm in diameter), meso- (5–25 mm in diameter), and micro-plastics (<5 mm in diameter) (OSPAR Commission, 2020). Macro- and meso-plastics extracted from collected biological samples, digestive contents or feces, need to be thoroughly cleaned to remove any remaining biological material before drying and storage in appropriate and labelled containers (see methods in Duncan et al., 2019a; González-Paredes, 2024; Provencher et al., 2019). While micro-plastics often need enzymatic digestion or treatment with potassium hydroxide (KOH) to remove organic material and biofilm (see methods in Duncan et al., 2019b; Joon Shim et al., 2017; Kühn et al., 2017). Care must be taken throughout the cleaning and storing micro-plastics to eliminate potential sample contamination. Among the most common sources of contamination are atmospheric contamination from airborne plastic particles, water contamination, equipment contamination and cross-sample contamination when multiple samples are being processed simultaneously (Bogdanowicz et al., 2021).

Incorporating the physical characteristics and chemical composition of plastics into assessments is critical, as these attributes may affect the impact of ingestion. One of the most established protocols for categorizing ingested macro- and meso-plastics by morphology and typology was developed by Van Franeker et al. (2011) for northern fulmars (*Fulmarus glacialis*) and later adapted for marine turtles by Galgani et al. (2013). Other characteristics related to impactability, such as flexibility, sharpness, texture or buoyancy could also be considered for a more comprehensive assessments (see methods in González-Paredes, 2024; Rizzi et al., 2019; Vélez-Rubio et al., 2018).

The colour analysis of ingested plastic is equally important, as it can influence ingestion selection, with certain colors resembling natural dietary items (Duncan et al., 2019a; Schuyler et al., 2014b). Classification is typically done by comparing the plastic colour to a standard chart of the visible spectrum, including black, white, and clear/transparent (see methods in Duncan et al., 2019a).

Polymer identification is also commonly performed to characterize ingested plastic across all size ranges, using techniques such as Fourier-transform infrared spectroscopy (FTIR) or Raman spectroscopy (see methods in Camedda et al., 2022; Digka et al., 2020; Jung et al., 2018). These techniques involve analyzing plastic samples with spectroscopy, in which the resulting spectrum is unique to each polymer, enabling identification by comparison with reference libraries. This method is also valuable for tracing and identifying potential sources of plastic pollution (Camedda et al., 2022; Jung et al., 2018; Rice et al., 2021).

Once ingested plastics are categorized and classified, they are subsequently quantified. This can be performed either as total ingestion per individual or according to the specific classification categories describe above. The most common reporting metrics used for quantification are (i) the number of plastic pieces (Nelms et al., 2016; Lynch, 2018; Moon et al., 2023, and references therein) and (ii) the mass of ingested plastic (Camedda et al., 2014; Matiddi et al., 2017; Domènech et al., 2019). While these metrics provide a general estimate of the amount of plastic ingested by turtles, they can introduce biases when assessing potential impacts. This is because plastic pieces vary in size, shape, and characteristics, each posing different risks to turtles. Similarly, ingested weight (dry or wet) may not accurately reflect the actual plastic load within a turtle's digestive system due to the varying densities of plastics. Alternatively, the volume of ingested plastic may serve as a more representative metric when evaluating the impact of plastic ingestion, particularly in cases of partial or total blockage of the digestive tract (González-Paredes, 2024).

Table 2 compiles references on the established methods and reporting metrics for analyzing ingested plastic samples (see Table 2).

2.3.2. Data analysis methods

The standardization and consistent use of protocols when collecting samples and datasets are crucial for ensuring data reliability, enabling meaningful comparisons across studies, and facilitating the identification of broader patterns and trends in plastic ingestion impacts on marine turtles. These practices are vital for ensuring quality assurance, maintaining objectivity, and reducing both systematic and random errors. Quality Assurance (QA) and Quality Control (QC) methods are widely used to enhance data integrity and ensure reliable scientific conclusions (Batini et al., 2009; Reynolds et al., 2011). While quality assurance (QA) applies in the research design process by deploying specific procedures to avoid jeopardizing the data collection methods, quality control (QC) refers to protocols focused on ensuring the integrity of collected data (see methods in Konieczka and Namieśnik, 2018). It is equally important to consider and report cases of individuals exhibiting no plastic ingestion to avoid overestimating ingestion rates (Lynch, 2018; Provencher et al., 2017). Turtles in which ingested plastic was not found after thorough analysis should be recorded as zero-plastic.

Analytical methods must be carefully assessed, and potential biases and constraints must be explicitly identified and considered to determine the most suitable analysis to derive meaningful insights from the generated dataset. Methods should be selected based on data type (quantitative or qualitative), research question(s) and project objective (s). Ultimately, the reliability, validity, and accuracy of the results discerned through the analytical method shall be evaluated for the quality assurance of the research conducted (see methods in Batini et al., 2009).

The available analytical methods are diverse, and the approach will depend on the research aim(s). In general terms, reporting rates of plastic ingestion from opportunistic or unstructured sampling applies a *descriptive approach* to examine the presence/absence, frequency of occurrence and incidence of ingested plastics using a cross-sectional strategy to gather the dataset (data collection in a particular point of time) (Barreiros and Barcelos, 2001; Digka et al., 2020; Stahelin et al., 2012). To assess the impact of plastic ingestion on turtle health, methods can be either quantitative or qualitative but applied using an *inductive approach* to evaluate and reveal patterns between and among variables and possibly deduce cause-effect relationships (Franzen-Klein et al., 2020; Rice et al., 2021; Wilcox et al., 2018). Monitoring plastic ingestion trends require quantitative analytical methods within an *inferential approach* to deduce patterns over time on a dataset collected systematically in a longitudinal manner (Choi et al., 2021; Domènech et al., 2019; Schuyler et al., 2014a).

2.4. Resources and capabilities

Assessing resources and capabilities is vital for ensuring the feasibility and success of research or monitoring initiatives. It identifies needs in personnel, funding, or equipment helping to set realistic scopes and timelines. This process optimizes resource allocation, considering related constraints and limitations. It can also highlight gaps requiring additional support or training, enhancing sustainability and long-term viability. By aligning resources and capabilities with objectives, it strengthens strategic planning and improves outcome reliability and quality.

2.4.1. Expertise

Research projects usually comprise different stages, which probably require different expertise. Hence, it is essential to ensure that appropriate expertise is available to complete all project stages, including design, approvals and permits, securing funding, using special equipment, data collection, data analysis and reporting.

Reporting plastic ingestion on opportunistic samples generally requires basic technical expertise. Monitoring ingestion trends over time requires a detailed understanding of the life stage and feeding strategy of the focal marine turtle species, as well as a higher level of expertise since monitoring process involves systematic sampling, continuous (re) assessment, and strong analytical skills to ensure reliable results. Assessing the impacts of plastic ingestion on turtle health requires an even higher level of expertise, including knowledge of veterinary medicine and pathology, to infer cause-and-effect relationships (Table 3).

2.4.2. Timeline

The project timeline should be pre-determined and scheduled according to the planned sequence of actions. It is crucial to consider both the overall time needed for completing the project and the time required for each stage, allowing for delays and contingencies (e.g., bureaucracy and permit approvals, availability of equipment and materials, weather constraints).

Setting a timeline is particularly important for monitoring projects, which require data to be collected for an appropriate period to enable thorough analysis and derive robust results from observed patterns and trends (Table 3).

2.4.3. Project budget

Financial planning is central to ensure that all research expenses and associated costs can be covered across all project stages for the entire project duration. Consideration should be given to the costs of human resources, field trips, materials and special equipment, advanced techniques, and infrastructure. In general, research projects involving longer temporal scales and established expertise require larger budgets (Table 3).

3. Future considerations

In recent years, there has been a notable increase in research efforts focused on plastic ingestion by marine turtles. Despite of this, there are still multiple knowledge gaps regarding their impacts on marine turtles that need to be addressed:

Standardization of methods: The threat of plastic pollution to marine turtles is being addressed from various perspectives, involving scientists and conservationists in research institutions or NGOs, as well as stakeholders and the general public through citizen science initiatives (Borrelle et al., 2017; Nelms et al., 2016). In this context, the consensus in the use of methods facilitates the comparison of results across studies, contributing significantly to a comprehensive assessment of this threat to marine turtles at a regional and/or population level (Darmon et al., 2022; Galgani et al., 2023; Fossi et al., 2018; Matiddi et al., 2019).

Systematic studies: Most assessments of plastic pollution threat are based on a relatively small number of turtles obtained opportunistically or as a by-product of studies where evaluating plastic ingestion was not a primary research goal (Casale et al. (2016), Lynch (2018) and references therein). Shifting towards well-structured and systematic studies, including representative samples of both dead and life animals across large spatial scales, would generate statistically robust sample sizes to enable broader impact assessments over time (Choi et al., 2021; Darmon et al., 2022; Domènech et al., 2019).

Assessing underrepresented species: Studies have been disproportionately focused on loggerhead and green turtles (Nelms et al. (2016), Lynch (2018), Moon et al. (2023), and references therein). To gain a more comprehensive and accurate understanding of interspecific variability in vulnerability, assessments of plastic ingestion should encompass all seven marine turtle species (Clukey et al., 2018; Duncan et al., 2019b).

Assessing underrepresented life stages: Turtle age/size classes represent another significant predictor of plastic ingestion (Nelms et al. (2016), Lynch (2018), Schuyler et al. (2014a) and references therein). The vulnerability of turtles across all life stages and habitats occupied throughout their lifespan needs to be determined. Special attention should be given to early life stage turtles as they are considered particularly vulnerable to the impacts of plastic ingestion, but also the most challenging to survey in the wild (Eastman et al., 2020; Pham et al., 2017; Rice et al., 2021; Ryan et al., 2016; Wildermann et al., 2018).

Population-level assessments: While the understanding of the individual-level consequences of plastic ingestion is extensive, significant gaps persist in evaluating the broader population-scale impacts (Fuentes et al., 2023; Nelms et al., 2016; Senko et al., 2020). Assessing the impacts of plastic ingestion on marine turtle populations is particularly challenging due to their complex life history and extensive distributions. Promoting collaborative research efforts is crucial for comprehensively assessing these impacts across populations and identifying hotspots within the regional management units for marine turtles (RMUs) (Wallace et al., 2010, 2023).

Evaluating underrepresented geographic areas: Marine turtles are globally distributed in tropical and temperate waters. However, most of the studies on plastic ingestion have occurred in the North Pacific, Atlantic and the Mediterranean (Nelms et al. (2016), Lynch (2018), Moon et al. (2023), and references therein). Expanding studies to under-researched geographical areas, including those within the Southeastern Atlantic, Pacific, and Indian Ocean, will provide a broader perspective

Table 3
Minimum resources and capabilities required to achieve research objectives when researching plastic ingestion in marine turtles.

Research objective	Expertise and research capability				Temporal scale	Budget
	[Logistics]	[Study design]	[Data collection]	[Data analysis]		
Descriptive reporting of plastic ingestion	Low	Low	Low	Medium	Low	Low/medium
Assessing health impacts of plastic ingestion	High	Medium	Medium	High	Medium/large	Medium/high
Monitoring incidence of plastic ingestion	Medium	High	High	Medium	Medium/large	High

TABLE KEY

Expertise
Low Achievable with minimal training
Medium Require specific training in the targeted field and/or in sample collection.
High Requires higher level training and could require input from external experts

Temporal scale
Small Month/s
Medium Months to Years
Large Years

Budget (USD $)
Low <$1000
Medium $1000–$10,000
High >$10,000

on the plastic pollution threat across different regional management units for marine turtles (RMUs). This is particularly important in highly polluted and/or under-researched geographical areas, aiming to identify plastic ingestion hotspots and population groups at risk (Duncan et al., 2021; Santos et al., 2021; Schuyler et al., 2014a, 2016).

Sub-lethal adverse effects: There is an extensive knowledge of the physical impacts caused by plastic ingestion in marine turtles (Nelms et al. (2016), Lynch (2018) and references therein). Nevertheless, the understanding of the sub-lethal effects associated with plastic ingestion remain unclear. Further research should be undertaken to improve our understanding of the impacts of plasticizers on the health of turtles (Sala et al., 2021; Savoca et al., 2023; Solé et al., 2022).

Multiple stressors: Marine turtles confront multiple threats across their life stages (Bolten et al., 2011; Hamann et al., 2010; Klein et al., 2017). However, current assessments are focused on isolated stressors, constraining broader analysis of the spatial and interconnected effects. Research efforts should prioritize assessing the cumulative and synergistic interactions of the multiple threats affecting marine turtle populations (Fuentes et al., 2023; Hart et al., 2018; López-Mendilaharsu et al., 2020).

4. Conclusions

Plastic ingestion is an ever-growing threat affecting all seven species of marine turtles. Understanding the full extent of the plastic pollution threat requires increasing research and monitoring efforts, as well as collaborative efforts among scientists, agencies, and stakeholders. Bringing together diverse disciplines such as biology, ecology, veterinary pathology, toxicology, and oceanography will allow a more comprehensive understanding of the threat process of plastic ingestion and its impacts.

Establishing a standardized framework that outlines best practices and research methodologies can unify research efforts and provide valuable guidance for stakeholders in assessing and monitoring plastic ingestion. By integrating this approach with open-access data repositories, the comparison of results will be facilitated, enhancing the broader evaluation of the impact of plastic pollution on marine turtles.

CRediT authorship contribution statement

Daniel González-Paredes: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft. **Emily Duncan:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Brendan J. Godley:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Helene Marsh:** Supervision, Writing – review & editing, Conceptualization, Validation. **Mark Hamann:** Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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