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Gastrointestinal transit times in juvenile green turtles: An approach for assessing digestive motility disorders

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ABSTRACT

The ingestion of anthropogenic marine debris can lead to injuries in the digestive system of marine turtles through blockages, lacerations and enteritis, as well as sub-lethal effects from bioaccumulation of adhered chemicals and toxic substances leached out into tissues and blood. The early detection of these impacts is central for the treatment and recovery of turtles in a rehabilitation setting. In this study, we provide baseline data on gastrointestinal transit times in healthy green turtles (*Chelonia mydas*) to enable non-intrusive detection of digestive motility disorders. We conducted two experiments with juvenile green turtles ($N = 14$) (curved carapace length range 33.7–47.0 cm) using inorganic (inert plastic discs) and organic (corn kernels) markers respectively, in order to estimate gastrointestinal transit times and assess the effectiveness of each marker type in recording them. Gastrointestinal transit times for the inorganic marker trial group ($n = 6$ turtles) ranged from 14.6 ± SD 3.6 days for the first markers recovered to 22.5 ± SD 4.2 days for the last markers recovered. The corresponding data for the organic marker trial group ($n = 8$ turtles) ranged from 6.63 ± SD 1.6 days to 17.3 ± SD 3.3 days respectively. We obtained 96% recovery success of markers in the inorganic marker trial versus 72.5% in the organic marker trial. Thus, inorganic markers proved to be more efficient in reporting gastrointestinal transit times because they do not degrade or discolour as they pass along the digestive process, enabling higher recovery success. Opportunistically, veterinarians diagnosed an obstruction caused by plastic fragments, which had been swallowed in the wild prior to the trial, in one of the experimental animals after we failed to recover any markers. This incident is evidence that gastrointestinal transit time assessment is a useful approach for providing early warning of digestive system blockages. Furthermore, this knowledge on transit times could be of interest for toxicology studies regarding exposure to chemicals lixiviated from debris ingested, as an index of the time spent by these substances inside the organism.

1. Introduction

The green turtle (*Chelonia mydas*) is a marine turtle species highly susceptible to human activities (Wallace et al., 2011). Currently, the species is listed at a global scale as Endangered on the IUCN Red List (Seminoff, 2004). The incidental ingestion of marine debris and fish hooks are among the recognised anthropogenic threats, causing injuries such as obstructions, lacerations and enteritis of the digestive tract, and potentially death (Wallace et al., 2013; Wallace et al., 2010; Schuyler et al., 2016; Schuyler et al., 2014; Wilcox et al., 2018; Santos et al., 2020; Santos et al., 2015; Eastman et al., 2020). In addition, although less is known, the ingestion of anthropogenic marine debris can lead to non-physical or sub-lethal adverse effects, resulting from chronical exposure to toxic substances leached out into blood and tissues (McCauley and Bjorndal, 1999; Clukey et al., 2018; Savoca et al., 2018; White et al., 2018; Sala et al., 2021).

These impacts from ingestion of marine debris are most commonly reported as internal injuries (Bugoni et al., 2001; Santos et al., 2015; Ryan et al., 2016; Vélez-Rubio et al., 2018; Wilcox et al., 2018; Franzen-Klein et al., 2020). While diagnosis of injuries can be assumed from

Abbreviations: CCL, Curved carapace length notch to tip; SCL, Straight carapace length notch to tip; JCU, James Cook University; UV light, Ultraviolet light.

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external or post mortem examination, accurate diagnosis in live animals generally involves costly and specialised equipment and techniques such as ultrasonic imaging, X-rays or laparoscopy (Herbst and Jacobson, 2003; Di Bello et al., 2006; Valente et al., 2008; Valente et al., 2007; Upite, 2011; De Majo et al., 2016). Nonetheless, there are conservation and rehabilitation organizations that do not have convenient access to these specialised equipment and techniques for accurate diagnoses due to budget constraints or proximity to facilities. Alternatively, assessing gastrointestinal transit times could be a reliable, non-intrusive and low-cost approach for detecting gastrointestinal motility disorders (Skoczylas, 1978; Spencer et al., 1998). The gastrointestinal transit time is defined as the time taken by an ingested item to pass through the entire digestive tract. These transit times have been successfully measured on several testudines species (Barboza, 1995; Taylor et al., 1996; Meyer, 1998; Spencer et al., 1998; Hatt et al., 2002), including marine turtles: loggerhead turtles, *Caretta caretta* (Di Bello et al., 2006; Valente et al., 2008); and green turtles, *Chelonia mydas* (Hadjichristophorou and Grove, 1983; Brand et al., 1999; McDermott et al., 2006; Amorocho and Reina, 2008).

In reptiles generally, gastrointestinal transit times vary according to digestive efficiency associated with specific feeding strategies: herbivory, omnivory or carnivory (Diaz-Figueroa and Mitchell, 2006).

For instance, Valente et al. (2008) recorded mean transit times of 13.2 ± 4.6 days in juvenile loggerhead turtles, a carnivorous species, while Amorocho and Reina (2008) recorded mean transit times of 23.3 ± 6.6 days in juvenile green turtles, a predominately herbivorous species. The loggerhead turtle shows higher digestive efficiency (Di Bello et al., 2006; Valente et al., 2008), which results in shorter gastrointestinal transit times. In contrast, the green turtle uses a hindgut-fermentation strategy to digest the structural carbohydrates in plant cell walls (Bjorndal, 1980; Brand et al., 1999; Mackie, 2002), and consequently presents longer gastrointestinal transit times.

Diet composition also influences gastrointestinal transit times within the same species. This is particularly interesting in green turtles due to the changes in their feeding behaviour and diet composition across their different life stages. Juvenile green turtles usually undergo an ontogenetic shift in diet once they move to neritic habitats at the completion of their pelagic life-stage. At that time, their feeding behaviour changes from opportunistic and omnivorous to primarily herbivorous, and from a pelagic to a benthic-based diet (Bolten, 2003; Reich et al., 2007; Arthur et al., 2008). However, some juvenile green turtles in temperate and subtropical waters forage omnivorously even in neritic habitats, indicating an adaptive capacity according to food availability (Arthur et al., 2008; Cardona et al., 2010; Cardona et al., 2009; González Carman et al., 2012; Gama et al., 2016; Vélez-Rubio et al., 2015, 2018, 2016).

Temperature also influences gastrointestinal transit times in reptiles. The metabolism of poikilothermic reptiles is regulated by the ambient temperature (Skoczylas, 1978; Williard, 2013). The optimal metabolic rate and highest digestive efficiency of green turtles occur within a specific range of temperatures, which Southwood (2003) experimentally estimated to be between 17 and 26 °C. At lower temperatures, turtles can remain active, but their metabolic rate decreases to a thermal threshold inducing dormancy. This thermal limit varies geographically among different green turtle aggregations, for example, 18 °C in Florida (Mendonça, 1983), 15 °C in the north-eastern Pacific Ocean (Seminoff, 2000), and 14 °C in south-eastern Australia (Read et al., 1996). The effects of higher temperatures on the metabolic rates of green turtles have been less studied. However, high temperatures are likely to lead to greater food intake and faster digestive rates as observed by Bjorndal (1980) in green turtles exposed to temperatures above 34 °C for long periods in the Bahamas during atypical El Niño years.

This study aims to generate baseline data by measuring the gastrointestinal transit times in juvenile green turtles. For this purpose, we assessed transit times in healthy animals using inorganic and organic markers, allowing us to compare their efficiency for such studies.

Additionally, we aim to validate the assessment of transit times as a non-intrusive complementary approach that could be used as an early warning sign of digestive motility disorders. Furthermore, knowledge of gastrointestinal transit times would inform studies on sub-lethal impacts caused by bioaccumulation of toxins leached out from anthropogenic debris ingested, as an index of the time that these substances remain within an organism.

2. Materials and methods

During 2019/20, two trials were conducted one each in Uruguay and Australia to estimate gastrointestinal transit times in juvenile green turtles. We used different type of markers in each trial, inorganic and organic, which allowed us to compare their efficiency in such studies.

2.1. Inorganic markers trial

Six green turtles were intentionally caught in the wild from Uruguayan waters using scientific capture techniques (see methods in Vélez-Rubio et al., 2016). All turtles were assessed by a veterinarian as being in healthy condition after capture, following standard procedures described in Eckert et al. (1999). These animals ranged in size from 33.7 to 47.0 cm in curved carapace length, notch to tip, (CCL) (mean ± SD = 40.6 ± 4.5 cm), and weighed between 4.4 and 10.9 Kg (mean ± SD = 7.5 ± 2.3 Kg).

The turtles were transferred to the Karumbé NGO rehabilitation facilities in La Coronilla (Rocha, Uruguay), and placed individually into 500 L tanks in a semi-shaded outdoor area. During the trial, the tanks were cleaned daily, and salt water was exchanged every three days. Water temperature and salinity were controlled to reflect natural conditions. Turtles were fed daily up to 10% of their body weight on a macroalgae *Ulva* sp., the main dietary item of green turtles in Uruguayan waters (Vélez-Rubio et al., 2016).

Prior the trials, turtles were allowed an adaptation period (6–8 days) under veterinary observation in order to detect any behaviour anomalies, and ensuring turtles ate regularly. On the first day of the trial, each turtle was given five purple markers made from 7 mm diameter discs of polypropylene (Alfepa Ltd., Uruguay). We administered them by intubating the turtles and introducing the markers one by one into the oesophagus with freshwater.

2.2. Organic markers trial

Eight green turtles, 32-months post-hatching, originating from Heron Island (Queensland, Australia) were kept in captivity in the Turtle Health Research Facility at James Cook University (Queensland, Australia). All turtles were under regular veterinary observation prior and during the trial period (following standard procedures described in Eckert et al., 1999). Additionally, we conducted blood analyses during the experiment, which reflected regular haematological values (Bolten and Bjorndal, 1992; Whiting et al., 2007; Flint et al., 2010). The turtles were assessed as clinically healthy with normal activity levels and behaviour. The animals ranged in size from 33.9 to 37.0 cm in CCL (mean ± SD = 35.9 ± 1.1 cm), and weighed between 4.4 and 5.3 kg (mean ± SD = 4.7 ± 0.3 kg).

Husbandry followed the protocols established by the JCU Turtle Health Research Facility, which is approved by the JCU Animal Ethics Committee in accordance with the Australian Code for the care and use of animals for scientific purposes. Animals were placed individually into 500 L and 1000 L tanks in a semi-shaded outdoor area. The seawater was sterilized by UV light and re-circulated through micro filters and fractionators for removing solids and oils. Water temperature and salinity were monitored during the trial period. Coprophagia was observed prior to the experiment and was considered part of the continuous foraging of the study turtles (Lance and Morafka 2001). In order to avoid re-ingestion of markers, we installed a mesh layer at the bottom of the tanks, facilitating faeces collection.

Turtles were fed daily at a rate of 4% body weight with a blended diet of vegetables, fish pellets, tinned sardines and vitamins (Sea Tabs®) compacted into gelatine cubes. On the first day of the trial, each turtle was fed 15 pre-cooked corn kernels (Coles Group Ltd. Australia), as organic markers, in batches of five with other food. According to prior observations when testing diet composition at JCU Turtle Health Research Facility (unpublished data), green turtles can ingest corn but do not easily digest it, resulting in whole corn kernels in their faeces.

2.3. Gastrointestinal transit time estimation

The monitoring tanks were checked several times each day for faeces collection. The presence and quantity of markers in each faeces were recorded. Turtles were monitored by veterinarians during the trial period at both locations in order to detect any anomalies in activity and feeding behaviour. After completing the trials, all turtles were released to the marine environment. Gastrointestinal transit times were recorded as the time between the ingestion and expulsion of the markers.

3. Results

3.1. Inorganic markers trial

The husbandry conditions reflected natural conditions in Uruguayan waters during the austral summer when the trial was conducted. The mean water temperature in the tanks was 23.4 ± SD 3.1 °C (ranged 16–32 °C); while the salinity averaged 30.5 ± SD 0.5. The changes in weight (gain or loss) of individual turtles during the trial period averaged 6%, ranging from 5% weight gain to 9% weight loss.

Markers were easily detected and recovered from faeces, recovery success was 96% (all the markers recovered, excepting the last one from turtle UY03). The gastrointestinal transit time for the expulsion of the first marker averaged 14.6 ± SD 3.6 days; and the transit time for the expulsion of the last marker averaged 22.5 ± SD 4.2 days (Table 1).

Turtle UY06 was excluded from the data analysis because none of the markers was recovered. The ingestion rate of this animal decreased until it stopped defecating on day six after starting the trial. The turtle was excluded from the trial and immediately transferred to the Karumbé rehabilitation area for appropriate treatment by veterinarians. A large amount of plastic were found in its faeces when defecation resumed (24 plastics fragments of different types, weighing a total 0.66 g). Subsequently, veterinarians diagnosed the individual with a partial obstruction caused by plastic ingested pre-capture while it was in its natural environment. We concluded that this obstruction was likely the main reason for the failed recovery of the markers during the trial period.

3.2. Organic markers trial

The husbandry conditions remained within the parameters established by the JCU Turtle Health Research Facility. The mean water temperature in the tanks was 27.7 ± SD 1.2 °C (range 24.1–30.0 °C); while salinity averaged 28.4 ± SD 0.2. The changes in the weight (gain or loss) of individual turtles during the trial period averaged 2%, ranged from 3% weight gain to 3% weight loss.

Markers were expelled in several defecations. Those expelled within the first 18 days were easily detected and recovered. However, markers expelled after >18 days were markedly degenerated by the digestion process and more difficult to detect and collect from the faeces. Overall recovery success was 72.5%. The total recovery per turtle averaged 10.9 ± SD 1.46 corn kernels of the 15 originally administered, ranging from 8 to 13 corn kernels per turtle.

The gastrointestinal transit time registered for the expulsion of the first marker averaged 6.63 ± SD 1.6 days; and the average transit time corresponding to the last marker recovered from each turtle was 17.3 ± SD 3.3 days (Table 2).

Transit times were expressed as the percentages of markers recovered in order to compare the results of both trials (see Fig. 1). We defined T1 as the time between the ingestion of the markers and the first defecation containing at least one of the markers; and subsequently T40, T60, T80 and T100 as the times required to expel 40, 60, 80 and 100% of the markers respectively. Turtles in the inorganic marker trial showed overall gastrointestinal transit times longer than turtles in the organic marker trial. We tested for potential correlations between the gastrointestinal transit times and data on temperature water, CCL and body mass, calculating Pearson's correlation coefficient in both experiments. Low or no significant correlation was observed between variables tested ($p > 0.05$). However, these results must be treated with caution due to the small sample sizes.

4. Discussion

The mean length of the transit time registered for inorganic markers ranged from two weeks (first markers expelled) to three weeks (last markers recovered). The corresponding data from organic markers ranged from one to two and a half weeks respectively. These findings fall within the ranges estimated for juvenile green turtles in previous studies (Table 3).

The differences in the results of these studies may result from experimental design and husbandry conditions. Furthermore, all previous studies indicated variations in the transit times within and between experimental animals. Authors attributed these variations to individual physiological and behavioural differences. This effect may also explain variations between individuals within our trials. Despite green turtles in trials of this study being smaller in size (carapace length) than those used in previous studies, there is no consistent evidence in the literature suggesting that digestive tracts of smaller animals, within the same species, would result in shorter gastrointestinal transit times.

Markers were expelled both individually and in batches in our trials. We assumed that markers travelled with food boluses as we also found all the markers attached to faeces. During digestion in green turtles, distinct boluses are compacted by the peristaltic movements of turtles' gut (Penry and Jumars, 1987; Bjorndal, 1997). Plastic markers are widely used for gastrointestinal transit time studies in reptiles (Hailey, 1997; Spencer et al., 1998; Brand et al., 1999; McDermott et al., 2006; Amorocho and Reina, 2008; Valente et al., 2008). They are low cost, or easy to manufacture, and do not suffer degradation or discoloration when passing through the digestive tract, making them reliable and easy to detect and recover. Apart from our organic marker trial, all the studies

Table 1
Biometry, husbandry conditions and gastrointestinal transit time for each marker expelled in the inorganic marker trial.

Turtle code	CCL (cm)	Weight (kg)	Water temperature, mean ± SD (°C)	Salinity, mean (ppt)	Gastrointestinal transit time (days)				
					1st marker	2sd marker	3rd marker	4th marker	5th marker
UY01	38.4	5.52	24.3 ± 4.2	30.0	19	19	20	22	23
UY02	47.0	10.90	25.6 ± 3.3	30.0	12	13	16	17	18
UY03	33.7	4.40	22.2 ± 3.6	31.0	11	11	11	13	–
UY04	40.8	7.40	21.7 ± 3.6	30.0	13	15	16	17	21
UY05	43.4	9.00	21.1 ± 2.9	31.0	18	21	21	25	28
UY06	40.2	7.73	20.3 ± 3.0	31.0	–	–	–	–	–

Table 2
Biometry, husbandry conditions and gastrointestinal transit time corresponding to each marker expelled in the organic marker trial.

Turtle code	CCL (cm)	Weight (kg)	Water temperature, mean ± SD (°C)	Salinity, mean (ppt)	Gastrointestinal transit time (days)				
					1st marker	2sd marker	3rd marker	4th marker	5th marker
JCU01	36.3	4.63	27.3 ± 1.1	28.6	6	6	10	11	17
JCU02	33.9	4.36	27.3 ± 1.1	28.6	5	6	6	8	9
JCU03	35.7	4.62	27.5 ± 1.0	28.3	6	6	6	6	6
JCU04	36.2	4.77	27.4 ± 1.0	28.3	6	9	9	10	10
JCU05	34.9	4.35	27.8 ± 1.0	28.4	9	9	11	11	16
JCU06	37.0	5.15	27.8 ± 1.2	28.4	9	9	9	10	10
JCU07	36.4	4.54	28.2 ± 1.4	28.3	7	14	14	14	14
JCU08	37.0	5.28	28.2 ± 1.4	28.3	5	6	6	11	11

Gastrointestinal transit time (days)									
6th marker	7th marker	8th marker	9th marker	10th marker	11th marker	12th marker	13th marker	14th marker	15th marker
17	17	17	18	19	19	–	–	–	–
10	10	10	17	20	20	–	–	–	–
6	7	8	8	8	8	11	14	–	–
10	10	11	11	11	21	–	–	–	–
16	16	17	17	17	–	–	–	–	–
10	10	10	11	16	16	20	–	–	–
14	14	15	–	–	–	–	–	–	–
12	12	12	12	12	12	–	–	–	–

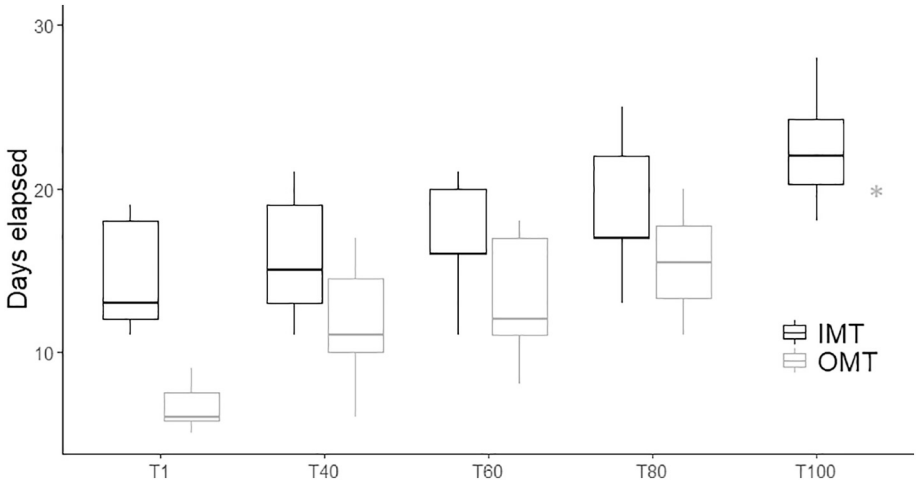

Fig. 1. Gastrointestinal transit times registered in the inorganic marker trial, IMT (black boxes) and organic marker trial, OMT (grey boxes). Values are expressed as the percentage of markers recovered. The intervals are defined as: T1, time for the expelling the first marker; T40, T60, T80 and T100 for times required to expel 40, 60, 80 and 100% of the markers respectively.
(*) No values registered for T100 in the organic marker trial.

Table 3
Experiment details and summary of results from studies of gastrointestinal transit times on juvenile green turtles (*Chelonia mydas*). Gastrointestinal transit time values are expressed as percentage of markers expelled; where Ti is the time to expulsion of the first marker, and Tf is the time to the last marker recovered.

Reference	Sample size (N = turtles)	CCL[1] range (cm)	Water temperature, mean ± SD (°C)	Transit times, mean ± SD (days)	Diet composition
McDermott et al. (2006)	4	60.6–72.7	26.6 ± 1.9	Ti = 15.4 ± 0.5 Tf ≥35	Natural diet; red alga *Gracilariopsis lemaneiformis*
Brand et al. (1999)	3	50.3–55.2	24.1 ± 2.4	T50 = 6.5–13.5[3]	Natural diet, free-range turtles
Amorocho and Reina (2008)	6	52.0–62.2 SCL[2]	28.3 ± 0.3	Ti = 22.0 ± 6.3 Tf = 24.7 ± 6.0	Maintenance diets; (a) fish based (b) plant based (c) fish & plant based
Inorganic marker trial (present study)	6	33.7–47.0	23.4 ± 3.1	Ti = 14.6 ± 3.6 Tf = 22.5 ± 4.2	Natural diet; green alga *Ulva sp.*
Organic marker trial (present study)	8	33.9–37.0	27.7 ± 1.2	Ti = 6.63 ± 1.6 Tf = 17.7 ± 3.7	Maintenance diet of mixed food

[1] CCL = curved carapace length.
[2] SCL = straight carapace length.
[3] Transit times reported in Brand et al. (1999) are given as a range of days for expelling of 50% of the markers.

in Table 3 used plastic markers. Nevertheless, potential secondary effects arising from the use of plastic markers such as chemical leaching are yet to be assessed. Organic markers such as corn kernels provide an alternative for assessing digestive motility in green turtles, avoiding issues of chemical leaching. However, corn is subject to degradation along the digestive process, which may hamper the detection of the last

markers expelled, and consequently reducing their recovery rate. We observed this disadvantage in our trials, reaching 72.5% recovery success using corn kernels as markers in comparison with 96% recovery success using plastic markers.

Water temperature is a factor that is likely to influence gastrointestinal transit times and trials should ideally be conducted in the wild (see Brand et al., 1999, and Amorocho and Reina, 2008); or on captive turtles maintained at temperatures reflecting natural conditions as much possible (see McDermott et al. (2006), and the inorganic marker trial in this study). Additionally, we postulate that the higher and more constant temperatures of our organic marker trial may partially explain the shorter gastrointestinal transit times showed by the study animals.

Another factor that is likely to influence gastrointestinal transit time is the diet administered during the experimentation. Green turtles are predominantly herbivores, using a hindgut-fermentation strategy (Bjorndal, 1980; Mackie, 2002). However, Higgins (2003) observed in long-captive green turtles a feeding adaptability to carnivorous or mixed artificial diets, which are commonly used in turtle rehabilitation programs. The mixed diet of processed food in the organic marker trial might have facilitated faster digestion in comparison to the diet based on macroalgae species *Ulva* provided in the inorganic marker trial, a result that may partially also explain shortened gastrointestinal transit times.

On the other hand, excess animal handling might increase stress and consequently affect digestive processes and gastrointestinal transit times. Valente et al. (2007) observed that stress caused by excessive handling in loggerhead turtle, *Caretta caretta*, induced longer gastrointestinal transit times. We minimised handling our experimental animals to reduce this factor as much as possible, aside from our need to handle turtles for administering the inorganic markers.

We were not able to recover any of the markers administered to turtle UY06 as this animal was diagnosed with a partial obstruction caused by plastic particles which we believe were ingested while the turtle was in the wild prior to the trial beginning. This incident is evidence that gastrointestinal transit time assessment is a useful non-intrusive and indirect approach for providing early warning of digestive system blockages.

In addition to the digestive motility disorders, there is increasing concern about the non-lethal impacts derived from anthropogenic debris ingestion, which could directly or indirectly lead to metabolic and endocrine malfunctions or fertility inhibition in males (Clukey et al., 2018; Savoca et al., 2018; White et al., 2018; Sala et al., 2021). The adverse effects caused by the lixiviation and absorption of toxic substances contained in, or adhered to, ingested debris is likely to be directly related to the time spent by these toxins inside the organism. Therefore, gastrointestinal transit times represent a parameter of relevance for future toxicology studies.

5. Conclusions

This study provides novel information on the gastrointestinal transit time on juvenile green turtles <50 cm in CCL, a class size for which there is no previous data. Ingested items can take from one week up to three weeks to pass through the entire digestive tract of a healthy turtle, assuming this does not get stuck along the process. After comparing the efficiency of both inorganic (inert plastic discs) and organic (corn kernels) markers recording transit times in our trials, we conclude inert plastic markers overall are more efficient since they not degraded or discoloured by the digestive process, enabling high recovery success. However, potential secondary effects such as chemical leaching should be considered. Other important factors affecting gastrointestinal transit times to be considered in experiments design include temperature and diet composition, which ideally should reflect natural conditions.

This baseline data on gastrointestinal transit times will contribute towards warning assessments of digestive motility disorders and toxicology studies on chemicals lixiviated from debris ingested.

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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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