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Ingesta passage and gastric emptying times in loggerhead sea turtles (*Caretta caretta*)

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Abstract

Ingesta passage times of soft flat foam dishes and gastric emptying time of barium-impregnated polyethylene spheres (BIPS®) were measured in 22 and 8 loggerhead sea turtles (*Caretta caretta*), respectively. Transit time (T_1) was considered as the time between ingestion and first elimination, and retention time (T_{50}) and total transit time (T_{85}) the expulsion time of 50% and 85% of the markers, respectively. The experiments were carried out at different times of the year and water temperature was recorded. A set of dorso-ventral radiographs was taken to locate the BIPS®, and the gastrointestinal anatomy of 5 dead turtles was studied to help with interpretation of the radiographs. No significant correlation was observed between T_1, T_{50}, T_{85} and minimum straight carapace length (SCLmin) or body mass and no statistical difference was found in ingesta passage transit times between juvenile ($n = 6$) and sub-adult turtles ($n = 16$). Mean passage times of the dishes (in days) were: $T_1 = 9.05$, $T_{50} = 12.00$ and $T_{85} = 13.19$. Gastric emptying time using BIPS® was 24–48 h. The transit time (T_1) for the BIPS® was longer (13.25 ± 4.86 days) than the foam markers (8.5 ± 2.73 days) in 8 turtles studied simultaneously. Although the total transit time tended to be faster in turtles submitted to water temperatures between 20 °C and 23.6 °C no significant correlation was observed between T_1, T_{50} and T_{85} and the temperature.

Keywords: Loggerhead sea turtle; *Caretta caretta*; BIPS®; Ingesta passage time; Transit time; Gastrointestinal tract morphology

1. Introduction

The loggerhead sea turtle (*Caretta caretta*) is a worldwide endangered species which is highly susceptible to human activity. The accidental ingestion of waste products from oil operations, plastic bags and other kinds of debris is one cause of mortality of this species throughout its distribution (Balazs, 1985; Bjorndal et al., 1994; Tomás et al., 2002; Milton and Lutz, 2003).

In some areas, such as the Mediterranean Sea, gastrointestinal disorders are common due to the ingestion of fishhooks, which cause traumatic injuries to the gastrointestinal tract leading to death (Pont and Alegre, 2000). Most turtles accidentally captured by fishing activities are released without removal of the hook. On admitttance to the marine rescue centres, diagnosis of gastrointestinal disorders in these turtles is very difficult; necropsy findings frequently reveal severe gastrointestinal injuries such as enteritis followed by necrosis, bowel obstruction and intussusceptions produced by the folding effect of the line pulling through the intestine (Orós et al., 2005; Di Bello et al., 2006a,b).

In domestic animals, knowledge of the ingesta passage and the normal gastric emptying times are useful in detecting gastrointestinal motility disorders and partial obstructions of the pylorus or small intestine (Manfred and Camilleri, 1992; Guilford, 2001). The digestive passage time of inert and indigestible markers, normally plastic pieces, or their simultaneous use with another type of marker have been used in many species, including chelonians (Lanyon and Marsh, 1995; Spencer et al., 1998; Hernot et al., 2006; Hailey, 1997).

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Barium sulphate suspension is the most frequently-used technique thanks to its inexpensiveness. However, it is not quantitative and evaluates the gastric emptying rate of the liquid but not the solid fraction of the ingesta (Guilford, 2001). When compared with a scintigram, the latter provides more accurate information (Goggin et al., 1998). Alternatively, barium-impregnated polyethylene spheres (BIPS®) have provided a range of diagnostic options which reduce the need to undertake exploratory surgery on cats and dogs (Nelson et al., 2001; Weber et al., 2002). The BIPS® are inert, white and have a density similar to food, but are sufficiently radiodense to show up clearly on abdominal radiographs.

In reptiles, mainly in herbivorous species, such as the iguana (*Iguana iguana*), Leopard tortoise (*Testudo pardalis*), desert tortoise (*Xerobates agassizii*), Galapagos Giant tortoise (*Geochelone nigra*), Greek tortoise (*Testudo hermanii*) and the short-necked turtle (*Emydura macquarii*), digestive studies have been performed to assess the normal transit and retention times and functional anatomy of the digestive tract (Barboza, 1995; Taylor et al., 1996; Meyer, 1998; Spencer et al., 1998; Smith et al., 2001; Hatt et al., 2002). The retention time in the aforementioned chelonians have varied from 7.5 to 14.8 days and were strongly influenced by both temperature and diet. Mean transit time of herbivorous species such as the Greek and Leopard tortoises were 2.6–17.3 h and 6–6.91 days, respectively (Taylor et al., 1996; Meyer, 1998). Hailey (1997) compared the digestive efficiency and gut morphology of omnivorous and herbivorous African tortoises (*Kinixys spekii* and *Geochelone pardalis*, respectively). The author found the transit time was lower in *K. spekki* (2.2 days) than in *G. pardalis* (3.8 days).

As to loggerhead sea turtles, Di Bello et al. (2006b) evaluated the post-enterotomy transit time of barium sulphate in the intestinal tract of six animals. Gastric emptying times and total digestive transit times were 34–264 h and 192–960 h, respectively. The authors stated that although often adequate for the diagnosis of obstruction, the procedures were not easily performed on some turtles.

The aim of the current study is to report on ingesta passage times in the healthy loggerhead sea turtles using specific indices such as the transit and retention times. Further, we have added the index T_{85} as 'total transit time' considering the long time of ingesta passage in chelonians. This work aimed to generate baseline data on digestive physiology, and to determine whether the established method of transit time assessment used in dogs and cats using BIPS® could be applied.

2. Materials and methods

Twenty-two loggerhead sea turtles (6 juvenile and 16 subadult specimens) accidentally caught in pelagic long line sets and fishing nets off the north-western Mediterranean coast of Spain were used in this study. Only those turtles in which the hook was superficially attached in or near the mouth and easily removed through the oral cavity were included in the study. Turtles showed minimum straight carapace length (SCLmin) of 31.5–54.5 cm and body weight of 4.4–22.2 kg. Juvenile turtles ($n = 6$) were considered to be those with a SCLmin of 21–40 cm and sub-adults ($n = 16$) those with a SCLmin of 41–65 cm (Dodd, 1988). The turtles were temporarily housed in the rehabilitation facilities of the Rescue Centre for Marine Animals (CRAM), in Premià de Mar, Barcelona, Spain. Complete blood count and serum chemistry values fell within normal limits. Only clinically normal animals were included in this study.

Turtles were accommodated in individual outdoor tanks measuring $100 \times 100 \times 50$ (deep) cm and were kept at the acclimatisation centre for a minimum of 2 weeks prior the study. The photoperiod ranged between 11 h/13 h and 13 h light/11 h dark according to season of the year. During the study, all turtles were fed at 48 h intervals with a diet based on hake (*Merluccius merluccius*) and sardines (*Sardina pilchardus sardina*) (1:1) in a quantity equivalent to 1.5–2.5% of the turtle body mass. No difference in the food intake was observed in the different seasons. Water temperature in the different tanks ranged according to the ambient temperature, and was measured twice daily (morning and afternoon), the mean values for each tank being used in the analyses.

Colour-marker experiments were carried out in 3 periods: early autumn, summer and winter, using 12 (8 subadults and 4 juveniles; mean body mass = 11.5 kg, mean SCLmin = 41.8 cm), 8 (6 subadults and 2 juveniles; mean body mass = 14.38 kg, mean SCLmin = 45.25 cm) and 2 turtles (2 subadults; mean body mass = 21.35 kg, mean SCLmin = 52.75 cm) respectively. We used different animals in each season. Each turtle, depending on its size, was given 10–20 coloured markers made with 5 mm of diameter dishes of soft flat foam (Evaland, Evapal® – Palencia, Spain) placed inside the fish at first feeding. The presence of the coloured markers in the faeces or floating in the water was recorded daily.

In the 8 turtles used in the study during the summer, BIPS® (Medical I.D. Systems, Inc.-Michigan, USA) were used simultaneously with the colour markers in the same animals to assess gastric emptying and digesta intestinal transit times. BIPS®capsules with two sphere sizes – 1.5 mm (30 U) and 5 mm (10 U) – were used. BIPS® and the colour markers were put inside of a mouth of one sardine that was swallowed by the turtle. Dorso-ventral radiographs were taken 2 h after the test meal to verify that all BIPS® were really swallowed. In the first week, radiographic monitoring was performed each 24 h. As no great changes in the position of the BIPS® were observed within the first 24 h, we decided to use a 48 h interval for subsequent radiographs for the remainder of the 21-day experiment. Lateral radiographs were extremely difficult to interpret because of the displacement and overlapping of the gastrointestinal tract with other soft tissue organs and carapace bony structure. Thus, all results reported are based on dorso-ventral radiographs only.

To determine the arrangement of the intestine and to identify the position of the BIPS® in the radiographs taken during the study, a preliminary anatomical study was performed in 5 dead juvenile turtles. Two cadavers were dissected, the arrangement of the intestines recorded *in situ,* and intestine length measured. The transition from ileum to colon was identified due to the presence of an iliocecal valve followed by a great enlargement of the colon, macroscopical changes in the colour and texture of the mucosa and a visible decrease in intestinal wall thickness (Wyneken, 2001). Three cadavers were frozen at −80 °C and sectioned with an electric bone saw in serial parallel sections between 18 and 20 mm thick, each one in an oriented plane (sagittal, dorsal and transverse).

Ingesta transit times are defined as follows: Transit time (T_1) the time between the ingestion of the coloured markers and the first defecation containing experimental material; retention time (T_{50}) and Total transit time (T_{85}) as the time required to excrete a minimum of 50% and 85% of ingested markers, respectively. Concerning BIPS®, T_1 was assumed once the first large size sphere (5 mm) was missing on the radiographs. We have added the index T_{85} instead T_{100} as ‘total transit time’ considering the long time of ingesta passage in chelonians. Gastric emptying time was defined as the time between first and last radiograph with BIPS® in the stomach area and was measured when all ten large BIPS® were out of the stomach.

Results – given as means ± s.d., n = number of individuals – were analyzed with STATISTICA for Windows (Stat Soft, Inc.). A Shapiro–Wilk test was used to verify the normality of data distribution. Linear regressions and Pearson’s Correlation were calculated between the T_1, T_{50} and T_{85} and temperature data, body mass and SCLmin. Graphics were done with Microsoft® Office Excel 2003. The best-fitting tendency line was used. Statistical differences for factors of variation such as kind of markers (colour markers versus BIPS®) and season (early autumn, summer and winter) were analyzed by a one-way ANOVA, a Levene Test for the homogeneity of variances, and a Scheffe test.

3. Results

The turtle dataset used in this study included some heterogeneous data because different juvenile and subadult turtles were examined in different seasons. Additionally, to evaluate the transit of the BIPS®, 8 turtles experimented in the summer must to be handled each 48 h for radiographic examination which might influence in the results. The stress level produced by this handling and how much it influenced the results could not be ascertained.

The data showed normal distribution. Coloured markers were expelled in several defecations and were easily visualized and recovered. In the study (using the foam dishes) with the 22 turtles, T_1 was 9.05 ± 3.05 (5–16) days, T_{50} 12.00 ± 4.53 (5–20) days and T_{85} 13.19 ± 4.64 (5–21) days. Low and no significant correlation was observed between ingesta passage times (T_1, T_{50}, T_{85}) and SCLmin or body mass ($p > 0.05$). No differences were observed between juveniles and subadults (Table 1).

The mean tank water temperature ranged between 16.27 °C (winter) and 23.86 °C (summer), with a range variation of as much as 3 °C in a same tank on a single day during summer. No significant correlation was observed between water temperature and T_1, T_{50} and T_{85} (T_1, $r = -0.24$ $p = 0.27$; T_{50}, $r = -0.16$ $p = 0.5$; T_{85}, $r = -0.26$ $p = 0.25$). No significant difference was observed among T_1, T_{50} and T_{85} of turtles studied in the three seasons ($p > 0.05$) (Table 2).

Table 1
Pearson’s Correlation between of the passage times (T_1, T_{50} and T_{85}) of coloured markers versus body mass (BM) and SCLmin and their comparison between aging groups of loggerhead sea turtles

Passage times (days)	Person’s correlations				Aging grouping		t	df	p	n-juv	n-sub
	BM	p	SCLmin	p	Juveniles	Subadults					
T_1	−0.20	0.38	−0.17	0.46	10.33	8.56	−1.23	20	0.23	6	16
T_{50}	−0.17	0.46	−0.12	0.60	12.83	11.67	−0.52	19	0.61	6	15
T_{85}	0.08	0.72	0.13	0.56	13.00	13.27	0.12	19	0.91	6	15

n-juv, n-sub: number of juveniles and subadults, respectively.

Table 2
Passages times (T_1, T_{50} and T_{85}) of coloured markers in the digestive tract of loggerhead sea turtles in different year seasons

Passage time (days)	Seasons			Variance			Scheffe test (p values)		
	Winter	Autumn	Summer	F-value	df	p	Winter × autumn	Winter × summer	Summer × autumn
T_1	13 ± 4.24	8.75 ± 2.86	8.5 ± 2.73	2.06	2	0.15	0.19	0.17	0.98
T_{50}	16 ± 5.66	11.91 ± 4.70	11.13 ± 4.12	0.93	2	0.41	0.52	0.42	0.93
T_{85}	20.5 ± 0.71	12.55 ± 4.84	12.25 ± 3.33	3.41	2	0.06	0.07	0.07	0.99
mT °C	16.31 ± 0.06	19.60 ± 1.74	23.86 ± 0.76	–	–	–	–	–	–

mT °C = Mean temperature of the water in the pools.

The quadratic regression curve was best fitted to the temperature data and ingesta passage times distribution. Total transit time tended to be faster in turtles submitted to water temperatures between 20 °C and 23.6 °C. The highest determination coefficient was observed when the total transit time (T_{85}) was plotted (Fig. 1).

All turtles showed the BIPS® in the stomach 2 h after ingestion. In this period, in 6 of the 8 tested turtles, the BIPS® were seen in the most caudal part of this organ (pyloric part). Gastric emptying time was 24–48 h. Large and small BIPS® moved in groups in the intestine (Fig. 2). However, in 2 turtles we observed that the BIPS® were retained for 48 h in specific parts of the digestive tract.

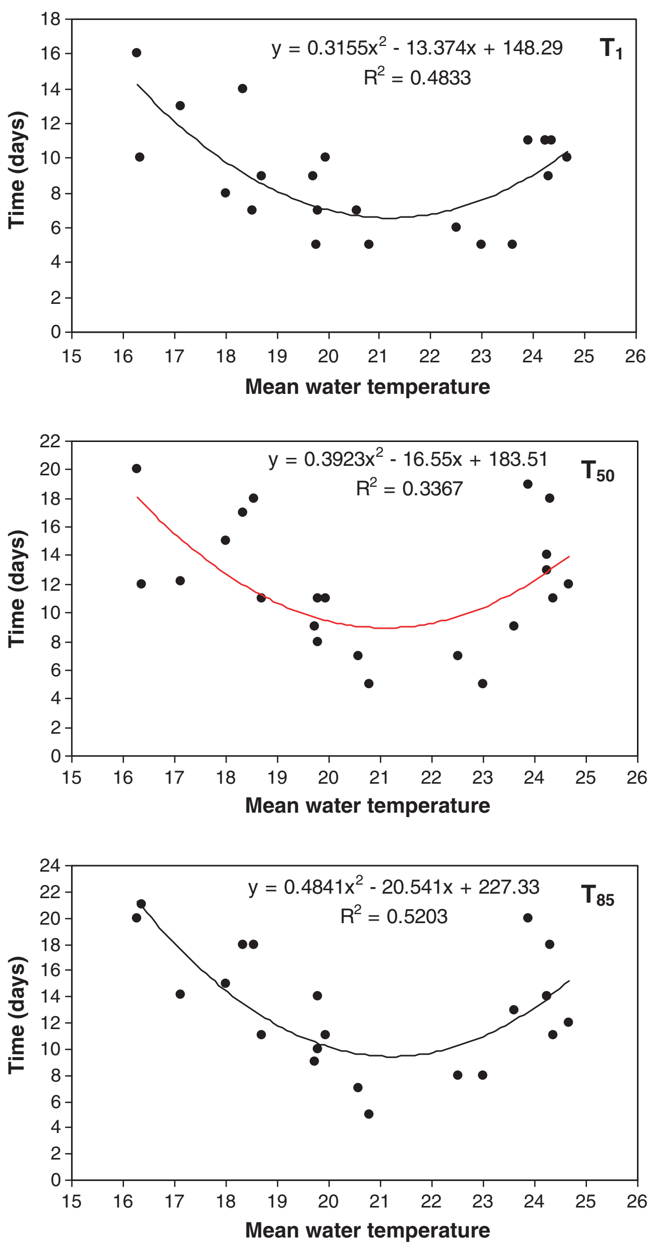

Fig. 1. Quadratic regression between ingesta passage times (T_1, T_{50} and T_{85}) using colour markers and mean tank water temperature of turtles studied.

In the radiographs, the exact positioning of the BIPS® in the intestine could not be identified and the orocolic transit time (usually calculated in dogs) could not be calculated. The superimposition of the carapace and pelvis with the small BIPS® made quantitative monitoring difficult.

In eight turtles, the BIPS® were expelled all together in T_1. In turtles tested simultaneously with BIPS® and colour markers, T_1 of the BIPS® was slower (13.25 ± 4.86 days) than the coloured markers (8.5 ± 2.73 days) ($p < 0.01$, $t = -3.56$, $\mathrm{df} = 7$). Eight of the 25 turtles retained some coloured markers and/or small BIPS® in their digestive tract at the end of the 23-day study period.

The anatomical study of the gastrointestinal tract in juveniles revealed that the small and large intestine were quite equal in length (turtle 1: SLCmin 31 cm, small intestine length 164 cm, large intestine length 148 cm; turtle 2: SLCmin 32 cm, small intestine length 135 cm, large intestine length 120 cm), with no apendicular cecal dilatation or structure. The large intestine (transverse colon) was divided into a dorsal and ventral part, resembling that of the equine large intestine (Fig. 2).

4. Discussion

An ideal marker is one that travels at the same rate as the food ingested. However, markers do not always move with the food or nutrients, making choice of marker one of the most important considerations in ingesta passage time studies (Robbins, 1993). Colour markers made from soft flat foam proved to be of great practical use in the sea turtle tanks. The major advantage observed in our study was ease of collection in faeces or in water due to floatability, which made accurate recovery and counting very easy. Sheets of ethyl vinyl acetate are inexpensive and are available in a wide variety of colours, being used widely in children's games thanks to low toxicity. The material did not lose colour in salt water and the markers could be manufactured using a standard hole punch.

In dogs, age affects T_{50} and orocecal transit time (OCTT). However, body size does not affect these parameters (Weber et al., 2003). Also, in Gopher and Galapagos Giant tortoises, retention time did not depend significantly on weight or age (Bjorndal, 1987; Hatt et al., 2002). Nevertheless in Galapagos Giant tortoises, it is interesting to note that the solid fraction of the digesta was retained differently in adult and juvenile tortoises (Hatt et al., 2002). In the current study, body mass and size of the turtle showed no significant correlation with the ingesta passage transit times and no differences in T_1, T_{50} and T_{85} were observed between juvenile and subadult specimens. In the live wild sea turtle, age (aging class) is usually estimated on carapace size and might not represent the real age.

Although described by the manufacturer as being 'low density', BIPS® were developed in view of the specific gravity of dry dog food, higher than the food of turtles. This was verified on expulsion in the defecation – the BIPS® sank to the bottom while the colour markers

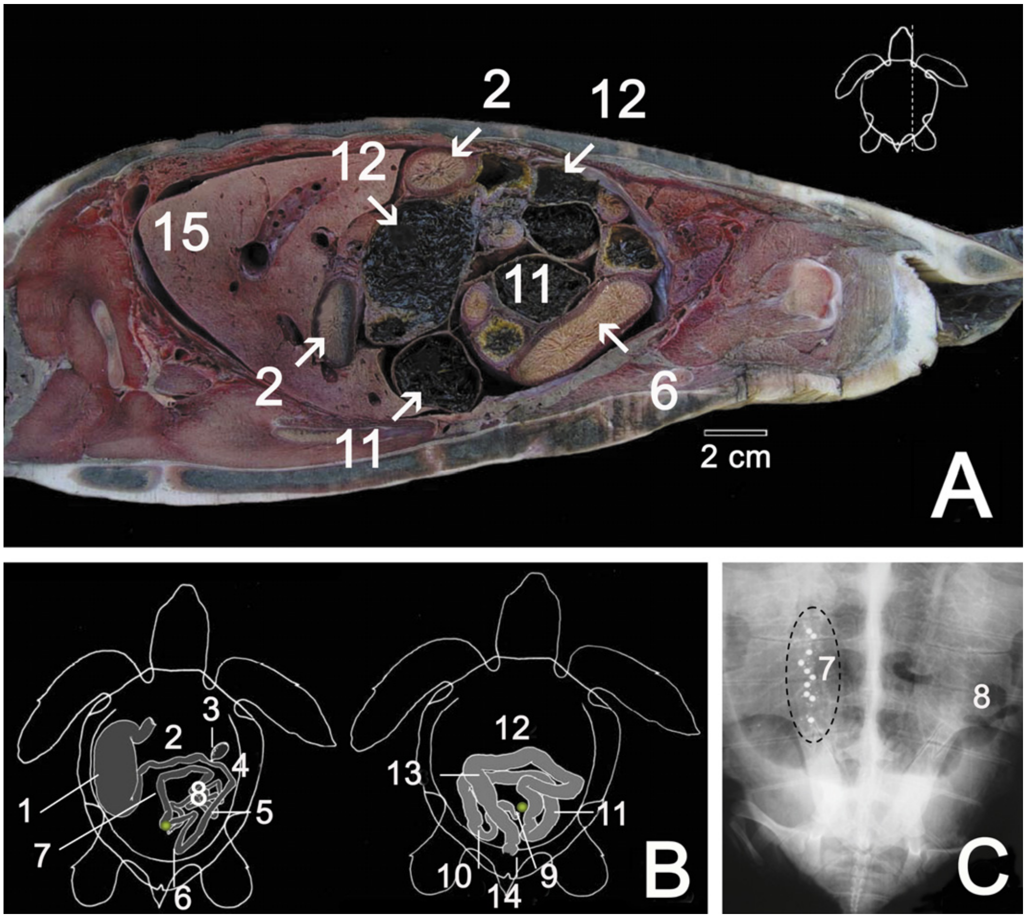

Fig. 2. Anatomical sagittal section of the juvenile loggerhead sea turtles (A) and drawing of the arrangement of the gastrointestinal tract (B): 1. stomach, 2. cranial part of duodenum, 3. gallbladder, 4. cranial flexure of duodenum, 5. descending duodenum, 6. caudal flexure of duodenum, 7. jejunum, 8. ileum, 9. ileo-colic junction, 10. ascending colon, 11. ventral transverse colon, 12. dorsal transverse colon, 13. descending colon, 14. cloaca, 15. liver. Dorso-ventral radiograph of juvenile loggerhead sea turtle studied with BIPS® (C): 7. Big and small BIPS® travelling in intestine, 8. loops of ileum.

stayed on the water surface together with the faeces. Due to their specific gravity, in sea turtles the BIPS® might travel together with the solid phase of the digesta whereas the very-low-density colour markers might travel with the liquid phase, thus accounting for the difference in evacuation time. In dogs, one set of dorso-ventral and lateral radiographs 6–24 h after BIPS® administration on an empty stomach is required to establish their position in the gastrointestinal tract. In our study we took radiographs after 2 h (to check that all markers were ingested) and after 24 h. As most of the turtles showed no changes in the BIPS® position in the first 24 h, we decided to increase radiographic monitoring to every 48 h thereafter. The ingesta transit times in the loggerhead sea turtle is longer than those reported for dogs and cats for which BIPS® are recommended (Weber et al., 2003). Therefore, in this species this method involves a high number of radiographs for each animal. This fact along with the limitations of the lateral radiographs and difficulties to address the right position of the BIPS® in the intestines do not justify its use in this species.

In our study, in a concurrent experiment, the transit time of the BIPS® was found to be longer than that of the colour markers. Previous studies comparing methods to evaluate retention time of digesta in lizards and tortoises have highlighted the importance of taking into account differences in the ingesta passage time and phase separation (solid and liquid), especially where transit time is long – an important digestive strategy in reptiles (Hatch and Afik, 1999; Hatt et al., 2002). A substantial difference in transit time using liquid phase markers has been observed in congeneric species of tortoises such as the *Testudo hermanni* and *T. pardalis* studied with gastrofin® and barium sulphate suspension respectively. A very fast transit time (2.6 h at 30.6 °C) was observed in Greek tortoises and a relatively slower transit time (about 6 days at 29 °C) in Leopard tortoises (Taylor et al., 1996; Meyer, 1998). The slow transit time in the Leopard tortoises could be associated with chemical immobilization carried out before the oral administration of barium.

The total transit time presented by Di Bello et al. (2006b) in six loggerhead sea turtles examined with barium sulphate administration three weeks after surgical procedure varied from 8 to 40 days. The gastric emptying time observed by these authors was much longer than that found in our study with healthy turtles.

The gut passage time is a reflection of gastric motility, in turn a reflection of health status, kind of food and ambient temperature of a reptile (Skoczylas, 1978; Spencer et al., 1998). Kind and frequency of feeding and water temperature of tanks were not mentioned by Di Bello et al. (2006b). The postoperative condition of the turtles in the study mentioned could imply gastric dysrhythmias, accounting for delay in gastric emptying time, as observed in mongrel dogs after undergoing abdominal surgery (Hotokezaka et al., 1997).

Compared with the mean total transit time (mean $T_{85} = 13.23$ days), the gastric emptying time (2 days) found in the loggerhead sea turtle seems to be relatively short. Radiographical and sectional studies in reptilians have shown that in omnivorous and carnivorous species the stomach is where the hold-up is longest, whereas in herbivorous species this appears to be in the caecum and proximal colon (Guard et al., 1980). Retention times observed in the loggerhead sea turtles in the current study were as long as those found in herbivorous chelonian species, such as the desert and Galapagos tortoises that experienced similar or even higher ambient temperatures (Bjorndal, 1987; Barboza, 1995; Hailey, 1997; Hatt et al., 2002). In reptiles, generally the herbivore intestine is longer than the omnivore intestine, and the omnivore intestine is longer than the carnivore intestine (Diaz-figueroa and Mitchell, 2006). The ratio of large intestine to small intestine lengths is significantly related to gut retention in omnivorous and herbivorous African tortoises (Hailey, 1997). The high ratio between large and small intestines observed in this study resembles those of tortoises, in which the large intestine is the section of the digestive tract with a great apparent area (Barboza, 1995). This finding could justify the relatively long retention time observed in the loggerhead sea turtles. Further studies concerning on morphology and digestive physiology are need in order to explain the function of the large intestine in this species.

The digesta transit times of turtles studied in different seasons did not show significant differences. These results disagree with those found in other chelonians (Meyer, 1998; Spencer et al., 1998). Concerning sea turtles, green turtles exposed to a laboratory simulation of subtropical winter and summer conditions showed relatively low thermal dependence of metabolic rate over the range of temperatures from 17 to 26 °C (Southwood et al., 2003). As to loggerhead sea turtles, a previous study performed in the Mid-Atlantic indicated that this species prefers to remain in areas with sea surface temperatures ranging from an average of 19.38–25.7 °C (Thomas and Dabo, 2005). The temperature range at which the turtles of our study tended to show fastest total transit time is placed just in the median of the preferred temperature range for the loggerhead sea turtles and could suggest the optimum or "comfort temperature" for this species.

Since in our study important factors such as acclimatisation, kind, amount and frequency of feeding were standardized, it is important to consider to what extent other captivity conditions could affect metabolism, breaking the positive correlation between temperature and metabolism known in reptiles (Gillooly et al., 2001). The ingesta passage times are proportionally related to the ambient temperature in reptiles (Skoczylas, 1978). Therefore, we would expect to find shorter ingesta passage times in turtles experimented in summer than those studied in autumn and winter which was not found in our study. On the one hand, results from winter could be influenced by the little number of animals studied which compromises the statistical results. On the other hand, the absence of differences in the ingesta passage times between summer and autumn could indicate that the water temperature range experimented in these season did not affect significantly the turtles' metabolism. The stress caused by the handling the turtles at much more frequent intervals to perform the radiographs and the rapid water overheating in the summer should be also considered to explain the tendency to increase T_{85} at higher water temperatures (Fig. 1). In this season, wide temperature range was observed in the tanks due to the small volume of water and the major effect of sunlight. The effect of intense heat as a potential stressor in juvenile and subadult sea turtles has not been studied. However, Gregory et al. (1996) found that small loggerhead turtles captured in the summer showed markedly higher corticosterone concentrations than those of large turtles captured in the same season and of all turtles captured during the winter, suggesting that a higher metabolic rate in this aging class due to higher water temperatures may be the basis for the variability of corticosterone between seasons. Sato et al. (1998) found that loggerhead sea turtles maintain body temperature higher than the ambient water temperature while swimming at sea, the difference being about 0.7–1.7 °C. In crocodilians, efficient temperatures for ingestion and digestion are between 25 °C and 35 °C; temperatures higher than 35 °C cause an undue amount of stress and inappetence (Lane, 2006). Like most reptiles, free-ranging sea turtles use behavioural means, such as postural adjustments or movements between different thermal microclimates, to maintain body temperature within a preferred range (Avery, 1982) which in our experiment was restricted by captivity conditions.

We believe that the new information provided in this study of ingesta passage time, the use of coloured ethyl vinyl acetate markers instead of BIPS® and knowledge regarding the effect of a excessive handling and comfort temperature of the loggerhead sea turtle may help rehabilitation centers and aquariums establish better conservation and management strategies, mainly in those turtles with digestive disorders or where there is a suspicion of the digestion of foreign bodies such as fishhooks.

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