



Experimental degradation of polymer shopping bags (standard and degradable plastic, and biodegradable) in the gastrointestinal fluids of sea turtles

Christin Müller^a, Kathy Townsend^b, Jörg Matschullat^{a,*}

^a Interdisciplinary Environmental Research Centre, Technical University Bergakademie Freiberg, Brennhausgasse 14, 09599 Freiberg, Saxony, Germany

^b School of Biological Sciences, Moreton Bay Research Station, The University of Queensland, P.O. Box 138, Dunwich, North Stradbroke Island, Queensland, Australia, 4180

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ABSTRACT

The persistence of marine debris such as discarded polymer bags has become globally an increasing hazard to marine life. To date, over 177 marine species have been recorded to ingest man-made polymers that cause life-threatening complications such as gut impaction and perforation. This study set out to test the decay characteristics of three common types of shopping bag polymers in sea turtle gastrointestinal fluids (GIF): standard and degradable plastic, and biodegradable. Fluids were obtained from the stomachs, small intestines and large intestines of a freshly dead Green turtle (*Chelonia mydas*) and a Loggerhead turtle (*Caretta caretta*). Controls were carried out with salt and freshwater. The degradation rate was measured over 49 days, based on mass loss. Degradation rates of the standard and the degradable plastic bags after 49 days across all treatments and controls were negligible. The biodegradable bags showed mass losses between 3 and 9%. This was a much slower rate than reported by the manufacturers in an industrial composting situation (100% in 49 days). The GIF of the herbivorous Green turtle showed an increased capacity to break down the biodegradable polymer relative to the carnivorous Loggerhead, but at a much lower rate than digestion of natural vegetative matter. While the breakdown rate of biodegradable polymers in the intestinal fluids of sea turtles is greater than standard and degradable plastics, it is proposed that this is not rapid enough to prevent morbidity. Further study is recommended to investigate the speed at which biodegradable polymers decompose outside of industrial composting situations, and their durability in marine and freshwater systems.

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1. Polymers in the marine environment and impact on marine biota

Plastics are synthetic organic polymers that exist for just over a century (Gorman, 1993). The material has become an essential part of modern everyday life due to its versatility and properties, such as low cost, adjustable transparency, light weight, strength and high durability. That durability may create a hazard at the end of the products' life cycle, however. This unwanted side effect is but one motivation to produce degradable and biodegradable polymers (also referred to as environmentally degradable) that would decay comparatively faster in the environment (Agamuthu and Faizura, 2005; ASTM, 1993; BiobagUSA, 2010).

Large amounts of plastic bags, styrofoams, rubbers, fishing lines and other hard-to-degrade plastic materials collect in the marine environment and may float for decades (Coe and Rogers, 1997). Derraik (2002) estimated that between 60 and 80% of all marine debris is plastic polymer-based. The source of this plastic debris is both land and sea-based. It has been estimated that ocean going vessels dispose

of between 4 and 6.5 million metric tons of plastic each year (Clark, 2001; NAS, 1975). More recent UNEP (2005) figures estimate a total of around 20 million metric tons of plastic from both land and sea sources, with land-based sources making up 80% of that total figure.

Marine debris has become a growing hazard for many marine animals. They mistake the plastic for natural prey items, ingesting it or becoming entangled within the rubbish, which reduces the overall fitness of the animal (Bjørndal et al., 1994; Page et al., 2004; Sheavly, 2005; Townsend, 2011). Laist (1997) estimated that plastic debris affects 267 species worldwide, including 86% of all sea turtles, up to 36% of seabirds, and up to 28% of all marine mammals. It is hypothesised that sea turtles have been impacted more heavily than other marine species as they have well-developed downward facing, keratinized papillae in their oesophagus (Townsend, 2011). The presumed function of these papillae is to trap food, while excess water is expelled prior to swallowing (Wyneken, 2001). This well-developed anatomical feature inhibits regurgitation and increases the probability that swallowed plastics remain within the digestive tract, causing gut impactions and/or perforations (Bugoni et al., 2001; Mader, 2006).

A wide variety of marine debris has been recovered from the intestinal tracts of sea turtles. In Florida it was found that of the 24 turtles autopsied, 71% had ingested plastic debris, another 38% contained

* Corresponding author.

E-mail address: joerg.matschullat@ioez.tu-freiberg.de (J. Matschullat).

monofilament fishing lines, 4% had ingested fish hooks, and also rubber, aluminum foil and tar were recovered from a small proportion of individuals (Bjorndal et al., 1994). Necropsies have found plastics within all sections of the turtle's gastrointestinal tract, including the oesophagus, stomach, small intestine, large intestine and rectum (Townsend, unpub. data). Other studies have also reported that plastic is the most ingested type of debris found within sea turtles (Balazs, 1985; Bjorndal et al., 1994; Bugoni et al., 2001; Plotkin and Amos, 1990; Sadove and Morreale, 1990; Shaver, 1991; Townsend, 2011).

Green turtles (*Chelonia mydas*) and Loggerhead turtles (*Caretta caretta*), the species studied in this work, are the most common species of turtles encountered in Moreton Bay Marine Park, Australia (Davie, 2011). Adult Green turtles are herbivores, while Loggerhead turtles are carnivores (Bjorndal, 1997). Enzymes in the acidic stomach milieu of herbivores are specialized in digesting plant-based material such as cellulose (Vahlteich, 1928). The breakdown of food continues under alkaline conditions in the small and large intestines, where the nutrients are absorbed. The digestion of plant-based nutrients (including cellulose and starch) occurs predominantly in the stomach and large intestine through both enzymatic and microbial processes, whereas the digestion of protein-based nutrients occurs mainly in the small intestine (Bjorndal, 1997).

1.1. Properties of standard, degradable and biodegradable polymer bags

Standard polymer bags, such as shopping bags from supermarkets, consist of polyethylene (PE) or polypropylene (PP), the most commonly used plastics. Polyethylene is composed of polymerized ethene molecules (C_2H_4). The material can be differentiated into different categories, based on density or molecular branching. Two types are important to produce plastic bags: low-density (LDPE) and high-density polyethylene (HDPE). Polypropylene consists of polymerized propene (C_3H_6) molecules. Both these plastics are not biodegradable; it may take centuries until the material effectively decays, mainly by UV-triggered photocatalytic disintegration (Greizerstein et al., 1993). Very recently, a much faster decay of plastic in the oceans was demonstrated (Saidu, 2009) – albeit irrelevant for the situation discussed in here.

Explicitly **degradable polymer bags** also consist of PE or PP, but contain additional substances like starch to stimulate degradation without the help of microorganisms. The decomposition begins with exposure to heat, UV-radiation or other stresses. Supposedly, such plastics even degrade underwater (d2w™, 2011). Polyethylene bags, however, do not degrade in composting situations, with experiments reporting negligible decay within 49 days under anoxic conditions (Greizerstein et al., 1993; Mohee et al., 2008). The bags disintegrate into smaller pieces, but do not dissolve completely. While remains might be less problematic for larger animals, they are more likely to be taken up by smaller ones (RMIT, 2003).

Biodegradable polymer bags are either made of plant-based materials such as starch or bio-synthesized (bacteria) materials. These polymers have been produced since 1990 (Mohee et al., 2008). The definition for biodegradable bags (Agamuthu and Faizura, 2005; ASTM, 1993) describes a degradable polymer in which the decomposition results from the action of bacteria, fungi, algae and other naturally occurring microorganisms. Biodegradables break down into H_2O , CO_2 , CH_4 , inorganic compounds and biomass (ASTM, 2003). The different processes of plastics' decomposition and biodegradation are depicted in Fig. 1. Degradation results from heat, moisture, sunlight and/or enzymes that shorten and weaken the polymer chains (Bidlemaier and Papadimitriou, 2000). It has been claimed that biodegradable bags can fully degrade within 49 days in industrial composting situations (BioBagUSA, 2010). Biodegradation results from metabolism of the material by a mixed microbial population in moist and warm environments. If only non-biological environmental agents can degrade the material, it is referred to as environmentally degradable plastic (EDP) – Agamuthu and Faizura (2005).

The aim of this study was to investigate the decomposition rates of these three different bag types in the gastrointestinal fluids (GIF) originating from the stomach, small and large intestines of an herbivorous Green turtle (*Chelonia mydas*) and a carnivorous Loggerhead turtle (*Caretta caretta*). We are further testing the hypothesis that the GIF harvested from an herbivorous Green turtle (*Chelonia mydas*) would be more efficient at breaking down the cellulose-based biodegradable bags than the carnivorous Loggerhead turtle (*Caretta caretta*). Generally, we are convinced that all types of polymer bags can degrade in both inorganic and organic processes – the differences in the materials degradation characteristics lie in the different time frames, and decay rates appear too slow to be of environmental relevance for most marine creatures.

2. Materials and methods

All bag material in this study was derived from standard shopping sources, readily available to the general public (Table 1). Gastrointestinal fluids were gathered from two freshly dead, stranded turtles, one Green turtle (*Chelonia mydas*; 43.9 cm curved carapace length, CCL) and one Loggerhead turtle (*Caretta caretta*; 92.6 cm CCL). Both specimens were collected at North Stradbroke Island and all experiments performed at the Moreton Bay Research Station. The Green turtle, a thin, weak specimen with an extremely small heart, was affected by a cardiovascular fluke (*Digenea Spirorchidae*; Glazebrook et al., 1989; Cribb and Gordon, 1998). The Loggerhead turtle was a healthy adult female that died strangled in fishing (crab pot) ropes. Both animals had died very recently. After the stomach, large and

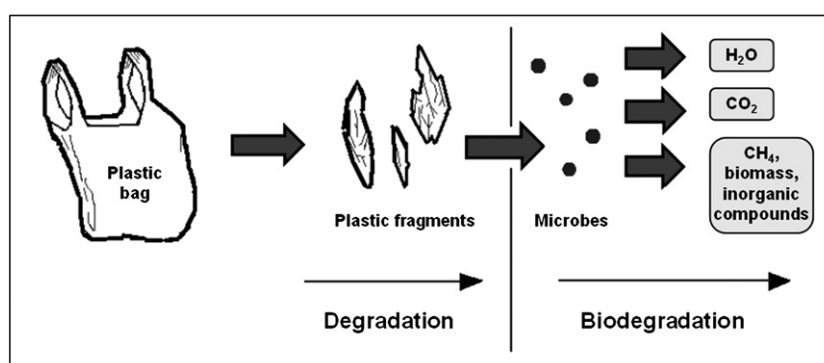


Fig. 1. Degradation and biodegradation processes (after Mohee et al., 2008).

Table 1
Types of plastic bags used in this study.

Type of bag	Characteristics
Normal plastic bag (no-brand)	HDPE-based, from Coles Supermarket
Degradable plastic bag	based on the d ₂ w™ technology which uses an additive combined with any blend of PE or PP
Biodegradable plastic bag	from BioBag®, claimed to be the first completely biodegradable and compostable bio-polymer. Consists of the Mater-Bi™ that comes from renewable raw materials of agricultural origin and from non-genetically modified starch (BioBagUSA, 2010). According to the manufacturer the bags are completely biodegradable in different environments: in composting, in the soil, in fresh and in salt water

small intestines were clamped off, the corresponding fluids were extracted into plastic containers, and stored directly in the refrigerator (5 °C). Prior to storage, pH values of the intestinal liquids were determined with litmus paper strips. The obtained pH values were between pH 7 and pH 8 for all three sections.

- For each turtle, the GIF, separately captured from each section of the gut, was divided into a set of nine 14 mL replicates and placed into labelled 15 mL PP centrifuge tubes. A further set of nine control liquid tubes containing salt water and freshwater were also prepared. Each of three different polymer bag materials were added separately to 3 replicates in each set, as strips with a size of 5 × 40 cm and a mass (t = 0) of approximately 300 mg. The tubes were protected against sunlight with duct tape and were moved continuously in an orbital mixer (RATEK Instruments PTY Ltd., Australia) to simulate gut contractions at a constant temperature of 24 °C (which is within normal internal spring/summer temperatures; Read et al., 1996). The “digested” plastic strips were weighed (Shimadzu AX 200; tolerance of ± 1 mg) every seven days for a total of 49 days. The strips were retrieved from the tubes, blotted dry and further dried very carefully under a fan. Following each measurement, the polymers were placed back into the tubes, which went back on the orbital mixer at randomized positions to the end of the experiments (49 days). Liquid loss was replaced by stored intestinal fluid.

The mean change in mass of the three polymer types over the 49 days for each gut section and between each turtle species was compared via a 2-tailed, paired t-tests using a statistical package from Microsoft® Excel 2002 (Data Analysis, t-test, paired two sample for means).

3. Results

The performed t-tests showed no significant change in mass for either the degradable or standard bags across all of the different fluids tested (including the controls; Table 2). Interestingly, a minor (non significant) weight increase (<1%) of the standard bag material occurred in the first three weeks of the experiment in the small and large intestine. This was also observed with the degradable bag in the large and the small intestine (1%) and may be explained by water sorption of the material. Further experiments would be necessary to better understand this phenomenon. The reference liquids

Table 2
p-values from a t-test for the measured variables (change in mass after 49 days, both species combined). Bold numbers represent the significance values where p ≤ 0.05.

Medium / Plastic type	Standard	Degradable	Biodegradable
Stomach	0.858	0.813	0.002
Large intestines	0.389	0.396	0.731
Small intestines	0.426	0.376	0.110
Saltwater	0.423	0.958	0.040
Freshwater	0.201	0.990	0.151

(salt and freshwater) showed minor weight variability (<1%), all within standard measuring inaccuracies.

However, significant differences did emerge for the biodegradable bags, particularly with stomach fluid and with saltwater (Table 2). This justifies an independent look at the degradation of the biodegradable bags for the two turtles (Fig. 2).

Overall, the change in mass of the biodegradable bag over the time of the experiment was higher for the Green turtle than it was for the Loggerhead turtle. The greatest losses were measured for the GIF in the Green turtle (≤9%). The large intestine follows (≤7% loss); small intestine, freshwater and saltwater showed a weight loss of barely 6%. Conversely, the highest mass losses were observed in the large intestine (≤6%) of the Loggerhead turtle. The small intestine and reference liquids yielded almost the same mass loss (4%). The lowest mass losses occurred in the stomach (≤3%) of the Loggerhead turtle. The degradation of the bio bag material in the GIF of the Green turtle stopped after 28 days (Fig. 2), while the degradation in the GIF of the carnivorous Loggerhead turtle stopped after one week.

Visual inspection of the test polymers showed that the biodegradable bags exhibited minor discoloration after the experiments, while no change was detected in the degradable or the standard plastic strips.

4. Discussion

This study emphasizes that biodegradable polymers react in very different ways in different mediums. Noting of course that the polymer bags were maintained in tubes that prevented both light and UV penetration, it is unclear if such light conditions also existed in industrial composting situation. This partly contradicts some industry information on the materials degradation characteristics. Tests run by BioBagUSA (2010) have found that the biodegradable bags used in this study can fully degrade (i.e. 100% mass loss) within 49 days in industrial composting situations. However, while the plastic and degradable bags showed no decomposition within the control

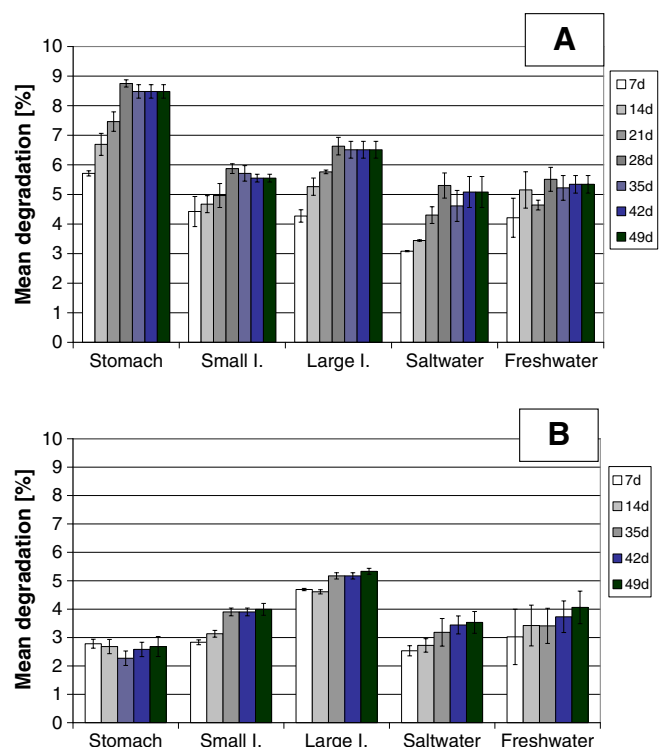


Fig. 2. Mean degradation, measured as relative mass loss (%) of biodegradable polymer bags in different gastrointestinal and control fluids (+/- std. error) for the Green (A) and Loggerhead (B) turtle.

mediums of fresh and salt water, biodegradable bags showed very low decomposition rates (approx 4.5%) after 49 days in salt water, or roughly 50% degradation after 389 days. This is a significantly slower rate than that found by BioBagUSA (2010).

Green turtles showed an increased ability to degrade cornstarch-based degradable bags throughout the entire gastrointestinal system, relative to the Loggerhead turtle and to the controls. This may be explained by the different diet of the two turtle species. Green turtles are herbivores with a gastrointestinal tract specialised to digest cellulose (Bjorndal, 1997). Thus, they may be more efficient in breaking down the bio-based polymers. However, the digestion rate of the biodegradable polymer bags (max 8.5%) in this study is much less than those seen in live Green turtles. For example, Amorcho and Reina (2008) have shown that equivalent sized Green turtles could digest up to 27% of a plant-based diet in 23.3 days (+/– 6.6 days). In contrast, Loggerhead turtles are carnivorous and have a much faster gut transit time of 24 to 48 hours (Valente et al., 2008), and may not be as efficient in breaking down cellulose polymers. This study shows that the maximum digestion of cellulose polymers in carnivorous alimentary systems occurs in the large intestine, while digestion in the stomach and small intestine do not exceed that of the control fluids freshwater and salt water. One reason may be the absence of related specialized enzymes.

5. Conclusions

Although biodegradable polymers may be an important improvement, their degradation in salt water is much slower than that claimed by the manufacturer (approx 3 years vs 49 days). This rate still represents a major improvement in the long-term (compared to thousands of years needed for standard plastics to degrade), yet these bags still pose a short-term threat to marine animals. Our study indicates that their breakdown in the gut is not fast enough to prevent morbidity and likely mortality of both Green and Loggerhead turtles. Hence, further studies are necessary to adequately assess the environmental decomposition of biodegradable polymers, particularly in sea water.

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