

Nancy

RECEIVED

JUL 26 1987

U.S. DEPARTMENT OF COMMERCE
BUREAU OF MARINE RESEARCH
WASHINGTON, D.C. 20540

*Turtles: debris
1501-22*

**Final Report for
U.S. Department of Commerce
& RFP No. FSN-5-0178**

Effect of ingestion of non biodegradable debris in sea turtles

Peter L. Lutz

Division of Biology and Living Resources

University of Miami

Rosenstiel School Of Marine and Atmospheric Science

4600 Rickenbacker Causeway

Miami, Florida 33149

TABLE OF CONTENTS

	<u>Page</u>
Abstract	3
Introduction	4
Literature Review	6
Materials and Methods	9
Results	14
Discussion	40
Summary	45
Literature Cited	47

Abstract

We observed that green and loggerhead sea turtles can actively consume plastic sheeting. But at the levels allowed in these experiments we detected no effects of plastic ingestion on feeding, gut function, metabolic rate, blood chemistry, liver function or salt balance. There was a change in blood glucose balance similar to, but much less than, that produced by starvation indicating a possible interference in glucose metabolism. It is suggested that blood glucose levels can serve as a sensitive index of nutritional status in sea turtles. Differences were found between green and loggerheads in feeding, absorption, hematocrit and white blood cell volumes. It is cautioned that the results should not be interpreted as evidence of the harmlessness of plastic ingestion by sea turtles, rather that this study should serve as a reference for further investigations on the effects the ingestion of higher, more realistic, levels of non-biodegradable materials by sea turtles.

Introduction

As man's use of non-biodegradable products increases, so does the amount of such material dumped in the ocean. Offshore garbage dumping by ships at sea is legal and the ocean is considered by some (Osterburg 1986) as "nature's trashbasket". However one consequence of this practice is that contact by marine animals with non-biodegradable refuse such as plastic bags and styrofoam products also increases. Recent incidents of entanglement and ingestion in marine mammals (Cawthorn 1985) and sea birds (Franeker 1985) have led to the belief that harmful contact with refuse may occur much more frequently than previously thought.

It is becoming increasingly recognised, that the ocean dumping of plastic waste in particular, presents a potentially serious hazard for sea turtles. Sea turtles consume a wide variety of debris and, in the man-made category, plastic bags and sheets appear to be the most prevalent material ingested (Balazs 1985). In some instances the level of contamination can be very high, for example plastic bags were found in 23% of a sample of green sea turtles in Peruvian waters (Hays and Brown 1981) and in one analysis 44% of adult non breeding leatherbacks were found to have plastic in their stomachs (Mrosovsky 1981). It has been suggested that one cause for ingestion is that turtles mistake the plastic for their natural jelly fish prey (Fritts 1982).

Is the ingestion of plastic by sea turtles any cause for concern? Clearly if sufficient plastic is swallowed to cause a complete stoppage of the gut death will result from starvation. However there are only a few

such documented cases (Balazs 1985, Cawthorn 1985) and most of the evidence for turtles swallowing plastic comes from butchered turtles (Balazs 1985). However, in vertebrates partial blockage of the intestine that is persistent can interfere with gut function (Fisher et al 1986) .In the sea turtle, plastic coating the gut walls could cause a reduction in gut absorption efficiency and also cause mechanical damage to the gut lining . Sub-lethal ingestion , therefore , where complete intestinal blockage does not occur , may be quite common and could adversely affect behavior , growth , reproduction and general homeostatic physiological function all of which may lead to other potentially lethal situations.

There is however , no information on the effects of sub-lethal ingestion of plastics by sea turtles . Given the critical position of most sea turtle populations and the huge magnitude of ocean dumping (van Dolah et al 1980 , Horsman 1982) it is clearly important to determine if the swallowing of such inert material by sea turtles is harmful and to establish the seriousness of any harm.

Literature review

There are few records in the literature concerning the impact of ocean debris on sea turtles. One of the earliest observations was made by A. Carr, who in the late 1950s noted a mass mortality of green turtles off the Costa Rican coast which he attributed to the ingestion of banana plastic bags (quoted in Cornelius 1975). The most recent and comprehensive report on the impact of ocean debris on sea turtles is the review by Balazs (1985) which provides a detailed case by case description of debris ingestion in different species. From this review it appears that plastic sheets or bags are the the major type of non-biodegradable material ingested by sea turtles and plastics have been found in the stomach or intestines of five species of sea turtle viz. green, loggerhead, hawksbill, leatherback and Kemps ridley.

There may be no single reason why turtles ingest plastic sheets. Swallowing plastic may be an accidental consequence of indiscriminate feeding. There is evidence that, with the exception of the leatherback, immature turtles show a tendency to feed on debris. For example, loggerhead hatchlings will eat anything that is floating and small enough to swallow (Hughes 1974). It has been suggested that floating plastic sheets may be mistaken for jellyfish and actively consumed, a process thought to be particularly important in the case of the leatherbacks, (Mrosovsky 1981).

Location is also important, for example Hays and Brown (1981) found plastic bags in 9 of 39 (23%) green turtles captured and slaughtered

off coastal Peru , while Mortimer (1981) found no sign of synthetic debris in the stomach contents of 243 greens captured in the Caribbean waters off Nicaragua . Although there is no direct evidence, it seems reasonable to imagine that ingestion would be more common in more highly polluted areas .In this regards particular concern has been expressed for Florida turtles since fish aggregating devices made of 6 ft vinyl screens are being anchored offshore (R. Whitham pers.comm.) and plastic sea weed mats are coming into common use to control beach erosion (van Dam 1984).

Leatherbacks may be the most at risk of all sea turtle species with regards to plastic ingestion . Jellyfish form a major part of the leatherback diet and probably for this reason leatherbacks have the highest incidence of swallowing plastic (Brongersma 1969, Hartog and van Nierop 1984) .In one analysis almost half (44%) of adult non breeding leatherbacks were found to have plastic in their stomachs (Mrovosky 1981) .Fritts (1982) found plastic bags and sheets in 13% of the leatherback carcasses collected in coastal Peru. Plastic was found in 7 out of 8 leatherbacks caught off the coast of France in 1980 (Duron and Duron 1980) and in 2 out of 3 caught in 1981 (Duguay and Duron 1982) . Brongersma (1982) noted plastic in the guts of two leatherbacks caught in European waters , Cawthorn (1985) recorded that a beached leatherback in New Zealand had its esophagus packed with polythene bags. .

There are reports of plastic sheeting being ingested in amounts sufficient to cause gut blockage and mortality (Hughes 1984, Duguay 1983) and the amounts can be high, sheeting as large as 3 x 4 m have been found in a leatherback (Hughes 1974). Although evidence for direct harm

resulting from the ingestion of lesser amounts is much less conclusive, Balazs (1985) is of the opinion that the potential problems are serious, resulting from a reduction in the absorption of nutrients and from the uptake of toxic plasticizers. He recommends that "studies should be conducted that involve the controlled feeding of plastics and other debris to turtles in captivity to gain definite information on intestinal obstruction, absorption of plasticizers, and feeding behaviour".

Materials and Methods

This is the first study of its kind and since there was no previous data to use as a guide and as we did not wish to cause any lasting harm to the experimental sea turtles we were particularly careful and cautious in designing our experimental protocol .

Animals

Green and loggerhead turtles were kept in tanks of approximately 1000 gallons capacity . Each tank was supplied with running , filtered sea water. The turtles were fed a specially formulated feed for sea turtles (Purina sea turtle chow) each day during the experiments unless otherwise noted.

Ingestion

The ingestion experiments were conducted in 3 sections . In the initial experiment green and loggerhead yearlings (approximately 1 kg weight) and juvenile (10 to 18 kg) turtles were allowed to consume a small single piece of plastic sheeting (10 x 10 cm) and were observed for about two weeks during which time various behavioral (yearling and juvenile) and physiological (juvenile) measurements were taken. The animals were fed turtle chow daily during the experiment. Since a preliminary examination of the data showed no adverse effects a second set of experiments was undertaken at an increased (but still modest) level of plastic ingestion . Seven loggerheads weighing 13 to 18 kg were used in this section (4 experimental , 3 control) and were fed 5 to 7 pieces of plastic sheet (10 x 10 cm) . They were also fed daily and

observed for two weeks. In these experiments the initial measurements, before feeding plastic, served as individual controls. In order to understand the effects of simple food limitation *per se* a third set of turtles were starved for two weeks and the various physiological parameters were monitored. This set also served as a control for those turtles in the previous experiments that occasionally refused food for a few days.

Gut function

Food consumption was measured as the number of pellets consumed each day. The pellets weighed on average 0.918 ± 0.085 g. Fæces were collected in plastic bags attached to the turtles and stored frozen at -20 C. It was noted that defæcation usually started 1 to two hours after feeding. Samples of food and fæces were dried at 67 C for 48 hrs and their calorific value measured using a Parr 1241 Adiabatic Calorimeter.

The ash content of food and fæces was estimated by weighing samples before and after being heated in a muffle furnace at 600 C for 24 hrs. Ash was used as a digestibility marker (Conover 1966). Although this method has been criticised because of its unproven assumption that ash forming materials are neither added to nor absorbed as food passes through the gut (Newman, Lutz and Snedaker 1985, Bjorndal 1985) it is fairly commonly used in studies of digestibility in marine organisms, and gives values in reasonable agreement with the acid insoluble method in sea turtles (Vargo et al 1986), it also has value as a comparative estimate.

Gut passage time was determined from the first appearance in the

fæces of the plastic sheets and of small plastic markers (teflon disks, 2-3mm diameter) that had been included in the food.

Occult blood in the fæces was tested for using the benzidine reaction (Henry 1974). Fæcal smears were taken and examined for the relative abundance of gram positive and gram negative bacteria.

Dive time

Dive time was recorded on a stopwatch while observing the turtles diving behaviour in the tank . Surface time was not measured since the interval was , almost without exception , less than 3 seconds (usually one breath).

Oxygen consumption

A closed circuit method was used for oxygen consumption measurements .The turtle was placed in a sealed humidified air chamber connected to an Applied Electrochemistry oxygen analyser. Chamber air was pumped through the analyser and returned to the chamber. Carbon dioxide and water vapour were removed for the analyser input line by chemical scrubbers (Ascarite and Dririte). The experiments were run for approximately one hour , and the minimal chamber partial oxygen pressures (pO_2) were always greater than 100 torr . Earlier experiments (Prange and Lutz 1984) and control experiments run in this study (fig 1) indicate that critical levels ,where the rate of oxygen consumption changes , are not reached until the air pO_2 reaches at least 15 torr . Using appropriate corrections the rate of oxygen consumption was calculated from the rate of fall in the chamber pO_2 .

Blood chemistry

Blood was taken from the dorsal cervical sinus as previously

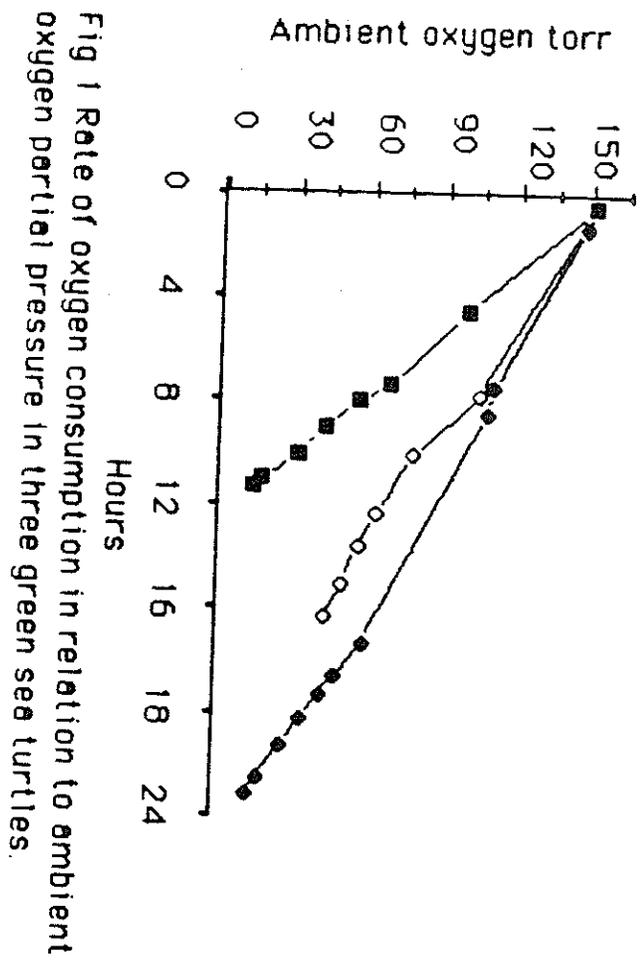


Fig 1 Rate of oxygen consumption in relation to ambient oxygen partial pressure in three green sea turtles.

described (Bentley and Dunbar-Cooper 1980).

Blood gases (pO_2 , PCO_2) and pH were determined immediately on whole blood using a Radiometer BMS Mk 2 blood - gas analyser set to the experimental temperature (22° C). Plasma bicarbonate was calculated from the pH and pCO_2 data using the temperature and pH dependent CO_2 solubility and dissociation constants of Severinghaus et al (1956).

The blood was then centrifuged and the plasma divided into two parts. One part was deproteinised with 8% chilled perchloric acid and served for plasma lactate and urea measurements using the Sigma kit no.826-uv for lactate and the Sigma kit no 640 for urea. The untreated plasma was analysed for osmotic pressure using a Wescor 6100 osmometer and saved frozen for measurement of ions and metabolites.

Plasma chloride was measured by an Aminco chloride titrator and plasma cations by atomic absorption spectrophotometry (Perkin Elmer PE 403).Column chromatography was used to estimate plasma cortisol and glutamic pyruvate transaminase levels were measured by spectrophotometry using Sigma kit no 505 .The hematocrit and the percentage volume of white blood cells were read after centrifugation.

Results

Feeding and digestion

Ingestion -

Ingestion of plastic sheeting introduced with food was seen in both species of sea turtle and in both age classes. The ingestion appeared to be of two types. Most commonly, the swallowing of plastic seemed to be accidental, occurring when a hungry turtle, during feeding coincidentally took in plastic intermingled with the floating food. On other occasions however, the plastic sheeting itself seemed to be the target for active ingestion. This behavior pattern was clearly seen when the plastic sheet was isolated several feet away from the nearest food item. In these circumstances the turtle appeared to swim directly to the floating plastic and on several instances we observed turtles having missed at the first attempt turn round and come back for another try. It should be noted, however, that for most exposures not all of the plastic offered was taken and a few pieces of plastic sheet were usually left in the tank after the food had been consumed. These were counted and removed.

Food consumption -

In the loggerheads daily food consumption did not vary much on an individual basis and when changes occurred they were fairly smooth (fig 2a). There was no noticeable pattern after feeding plastic. The average daily rate of consumption (g food /kg body weight /day) for individual loggerheads was $5.07 \pm 1.97, n=7$; $5.9 \pm 3.08, n=7$; $9.2 \pm 1.59, n=11$; $9.3 \pm 2.06, n=8$. In the greens the the average rates were similar ie $6.7 \pm 3.8, n=8$; $10.9 \pm 1.93, n=8$; $11.82 \pm 2.8, n=9$. However in one of the greens consumption

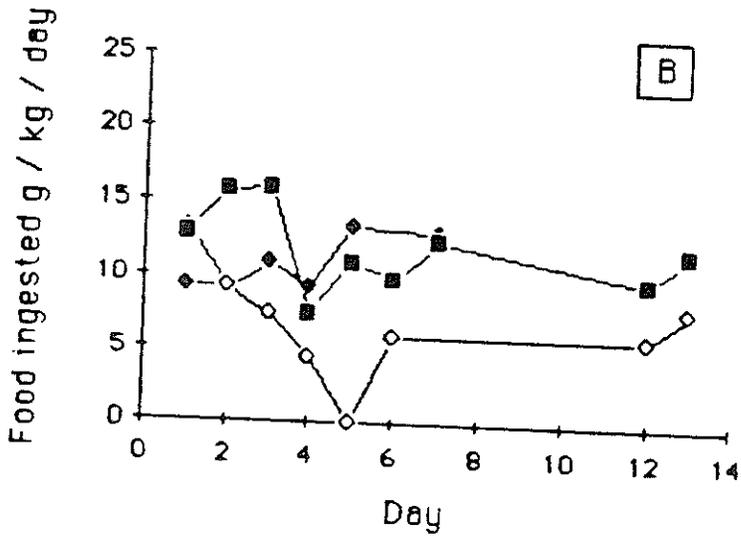
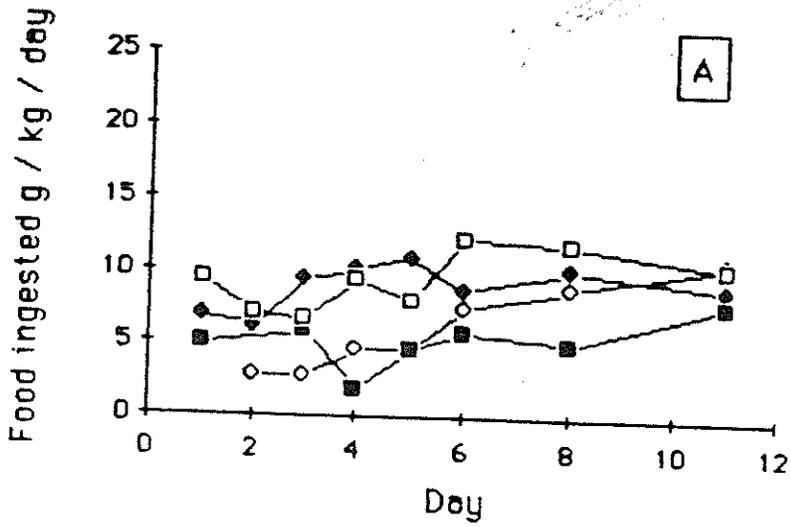


Fig 2 The effect of plastic ingestion (day 1) on food consumption in the loggerhead (A) and in the green (B) sea turtles.

gradually diminished to zero on day 4 and then recovered (fig 2 b).The consumption pattern for the other two turtles was similar to that observed in the loggerheads.

Gut passage time -

The gut passage time for the turtles fed pellets mixed with inert markers was 11.3 days (range 10 to 13 days , n=3). This value is almost identical to that found for loggerheads fed plastic (10.5±1.41 days , n=8).

Food absorption -

There was no change in the efficiency of digestion of the turtle chow following plastic ingestion .Digestibility ranged between 55 and 70 % throughout the course of the experiment (fig 3).The efficiency of digestion of the organic material in the food , a more realistic indicator of gut functioning , also remained within narrow bounds (60to 75% ,fig 4).

Energy adsorption -

The calorific value of the faeces showed no consistent change with time in either the greens or the loggerheads (fig 5). Interestingly the green faeces had a higher calorific content than the loggerhead (loggerhead faeces 3328 ±145 cal/g ,n=10; green 4126±324 cal/g,n=9). These differences were statistically significant (p < 0.01). It can be calculated that an amount loggerhead food containing 1g of ash would have a gross energy content of 51381 cal while faeces with the same amount of ash would have 12743 cal. Assuming constancy of ash, this indicates a digestible energy adsorption efficiency of 75.2% .

Stool culture

In the two control loggerheads the fecal flora was respectively 99% gram

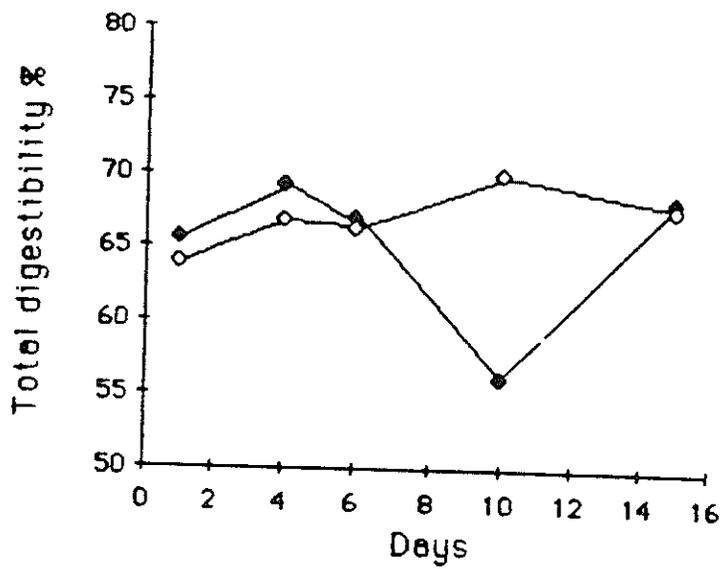


Fig 3 The effect of plastic ingestion on total digestibility in the loggerhead sea turtle

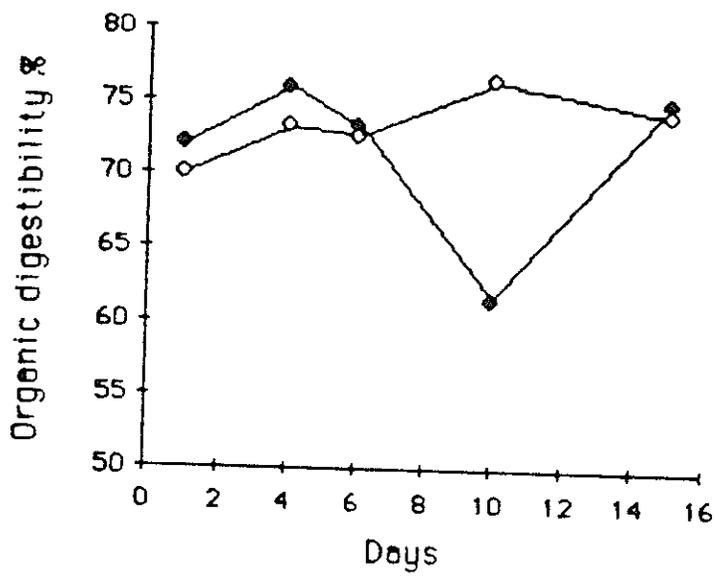


Fig 4 The effect of plastic ingestion on organic digestibility in the loggerhead sea turtle

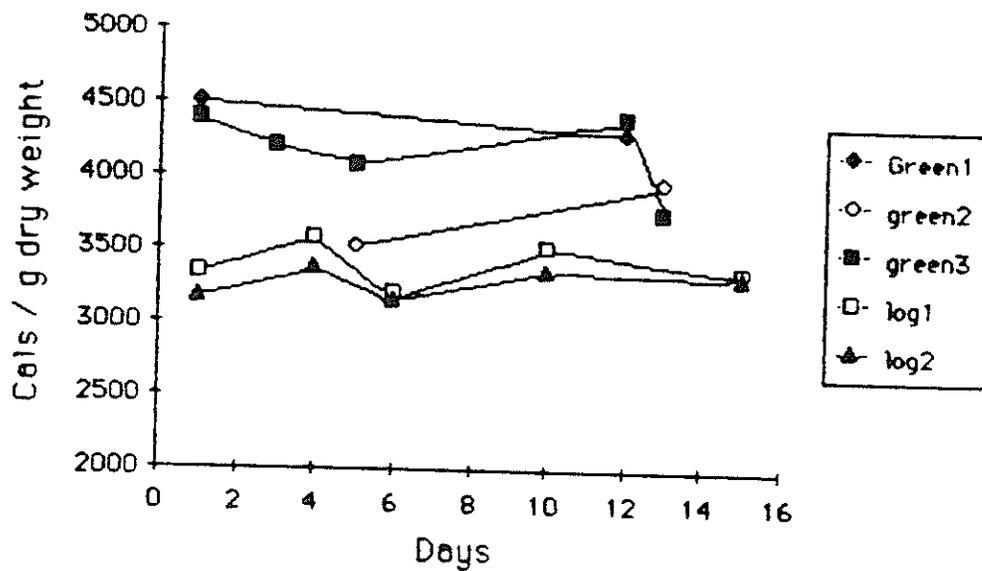


Fig 5 The calorific value of green and loggerhead turtle faeces after being fed plastic sheeting (day 1)

positive , 1% gram negative and 98% gram positive , 2% gram negative. In three turtles that had been fed plastic bags the faecal floral composition was as follows- 100% gram positive; 85% gram positive, 15% gram negative; 100% gram positive .The gut bacterial composition was therefore substantially gram positive in nature and this feature was not altered by plastic ingestion.

Occult blood

No occult blood was observed in any of the faecal samples examined either in the control or the experimental. The plastic ingestion ,therefore ,had not caused intestinal bleeding.

Respiration

Dive time-

The mean dive times before plastic ingestion ranged from 121 to 145 secs in the green and 90 to 114 secs in the loggerhead (fig 6 a and b) . Although the mean values declined in the first and second day after ingestion (green - day 1: 75 to 92 secs , day 2 : 52 to 62 secs ; loggerhead - day 2 : 72 to 78 secs ,) the reductions were not significant.

Oxygen consumption -

Plastic ingestion had no apparent effect on the oxygen consumption of both the green and loggerhead turtles, and on an individual basis they were remarkably constant over the two weeks of monitoring (fig 7) . Metabolic rates for the greens ranged from 47.9 to 73.8 ml/kg/hr and , with the exception of a single high reading for loggerhead * 1 on day four, the loggerhead values showed an almost identical range of from 38.1

100

100

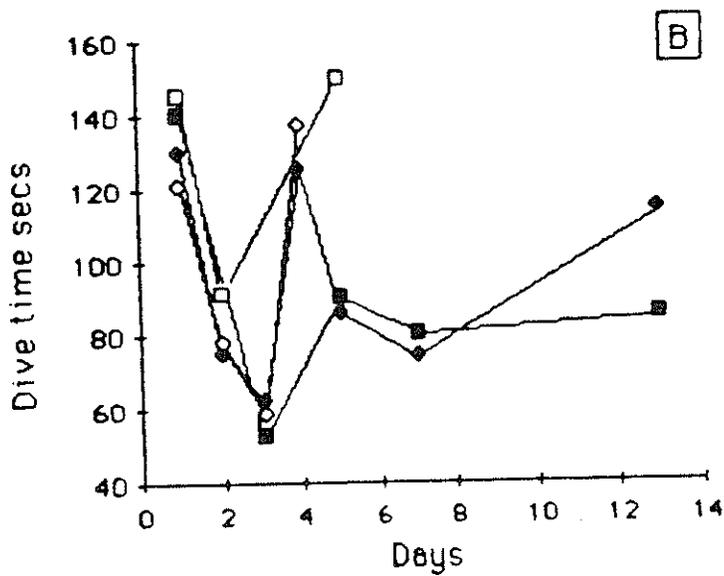
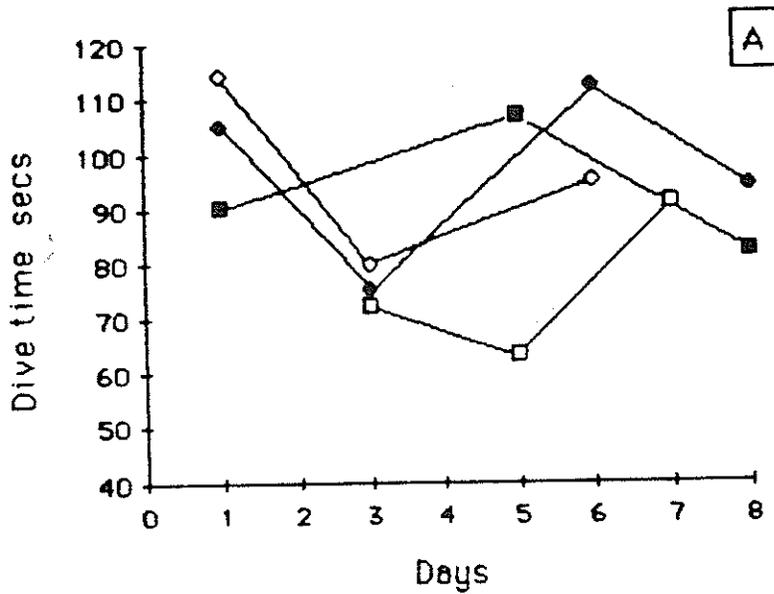


Fig 6 The effect of plastic ingestion (day 1) on dive time in loggerhead (A) and green (B) sea turtles

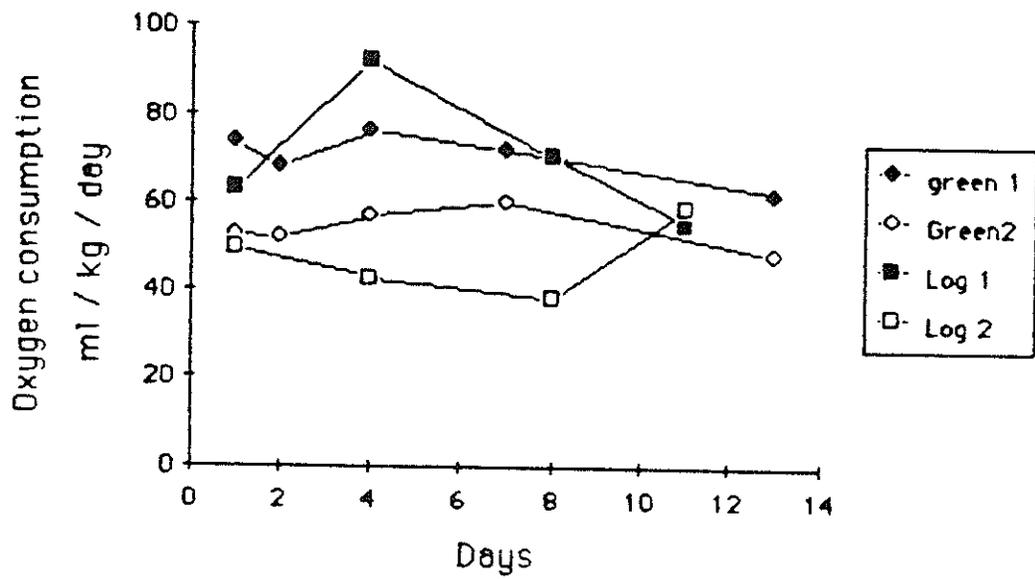


Fig 7 The effect of plastic ingestion (day 1) on oxygen consumption in green and loggerhead sea turtles.

to 70.2 ml/kg/hr. Similar oxygen consumptions have been obtained for greens (70.8 ml/kg/hr at 25 °C , Kraus and Jackson 1985) and loggerheads (62.0 ml/kg/hr , Lutz and Bentley 1985) measured in air.

Blood gases and acid base balance

Oxygen -

Venous oxygen levels remained relatively constant in both the experimental turtles and in the starved group (fig 8 a and b) , there was no significant difference between groups . Since venous oxygen levels are determined by the difference between oxygen supply and tissue use and since oxygen consumption did not change (see above) it seems likely that the mechanisms for oxygen transport have not been affected by plastic ingestion. The mean value for all of the data ($pO_{2v} = 56.69 \pm 1.59, n=38$) is very similar to that found in an earlier study on the same animals (Lutz and Dunbar-Cooper 1987).

Carbon dioxide -

Venous carbon dioxide remained similarly constant over the course of the experiment and no statistical difference was found between the control and the experimental groups (fig 9 a and b). The mean value for all of the data ($pCO_{2v} = 24.79 \pm 0.976, n=38$) is somewhat lower than that obtained earlier (about 30 torr at 20° C, Lutz et al in press).

Blood pH

For the group of experimental turtles fed plastic venous blood pH appeared to decline on the first day after feeding plastic ($p < 0.5$) and continued to fall in two turtles on day two and in one until day three (Fig

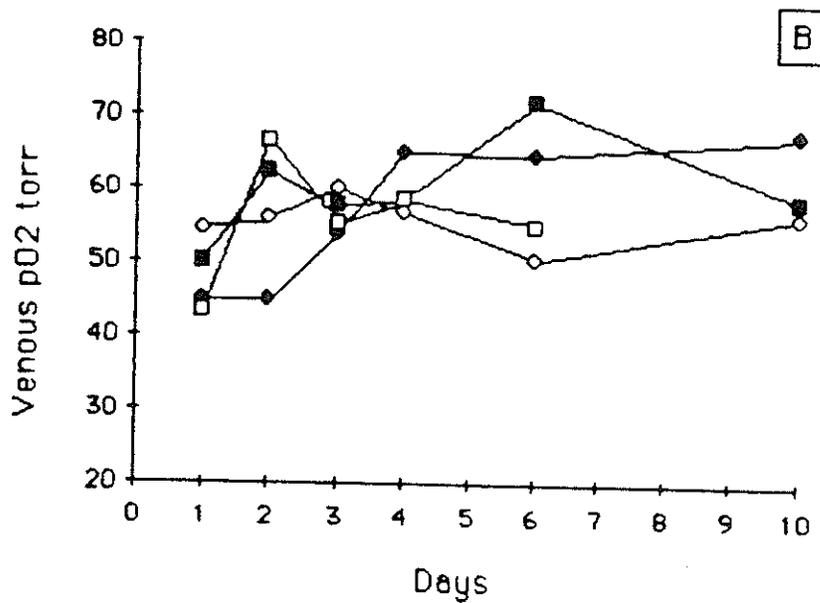
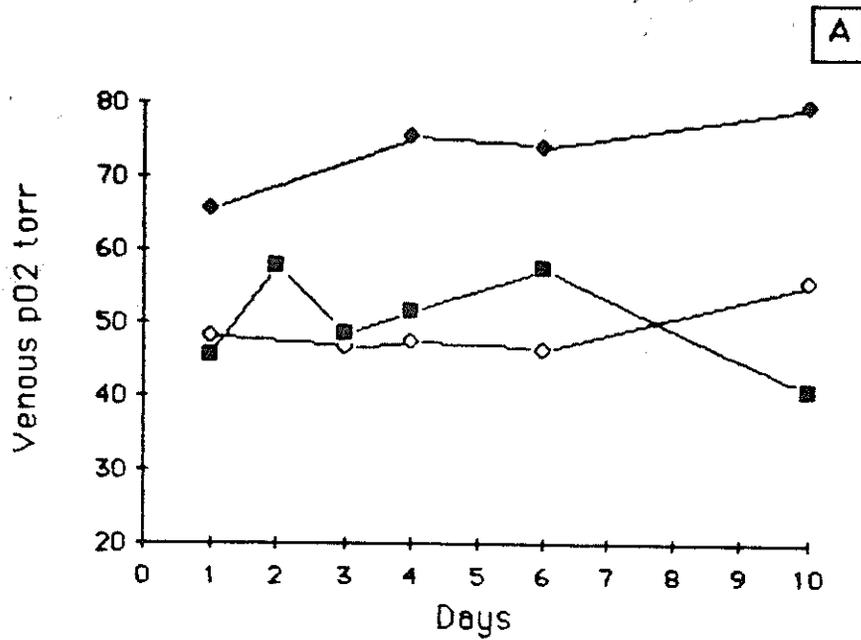


Fig 8 Changes in venous pO₂ in the loggerhead turtle during starvation (A) and following plastic ingestion (B).

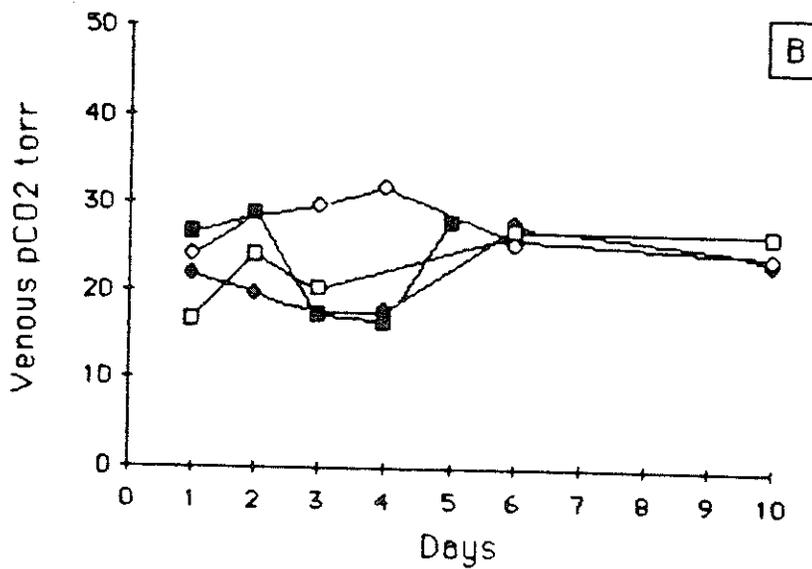
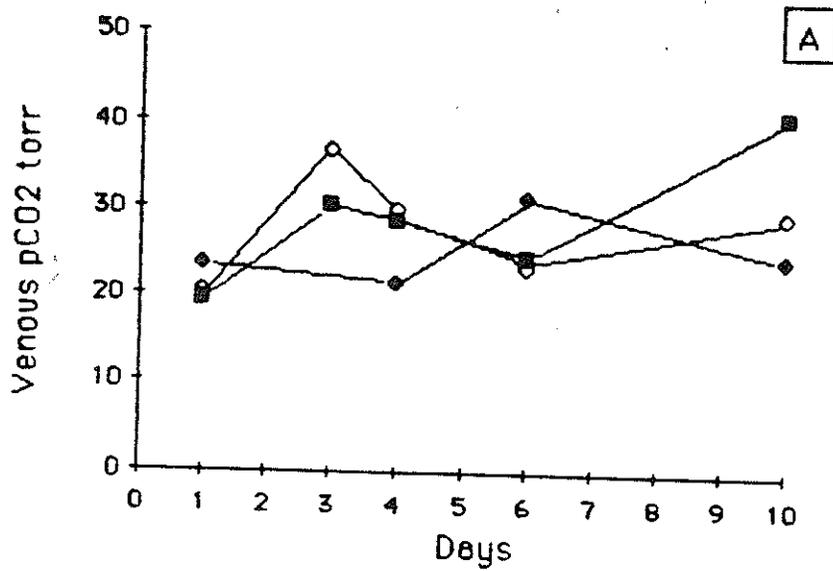


Fig 9 Changes in venous pCO₂ in the loggerhead turtle during starvation (A) and following plastic ingestion (B).

10a). No such trend was noted for the starved controls (Fig 10b). However the range in pH shifts was very narrow and for the whole set the average pH was 7.550 ± 0.008 , $n = 38$, close to the predicted normal venous pH for the prevailing body temperature (25°C , $\text{pH} = 7.442$, Lutz et al in press).

Bicarbonate -

While there was no change in venous bicarbonate on the day following plastic ingestion, in three turtles (but not in a fourth) bicarbonate declined on days two and three (Fig 11a). Again no similar trend was noted in the starved controls (fig 11 b) but the differences were not statistically different. The overall bicarbonate concentration was 22.6 ± 0.971 mM, $n=38$.

Blood chemistry

Sodium -

Although there was some individual variation, plasma sodium showed no particular trend in either the greens or the loggerheads fed plastic or in the starved loggerhead controls (figs 12, 13 a and b).

Chloride -

The plasma chloride values showed a greater range in those turtles that had eaten plastic compared to those fed on turtle chow alone.

However on an individual basis the values were reasonably constant and there was no noticeable difference between the two groups (Fig 14a and b).

Osmotic pressure -

The plasma osmotic pressure was remarkably steady in all groups tested, ranging from 320 to 350 mOsm (fig 15 a and b). These values agree closely with those found for the wild population (321 mOsm, Lutz and

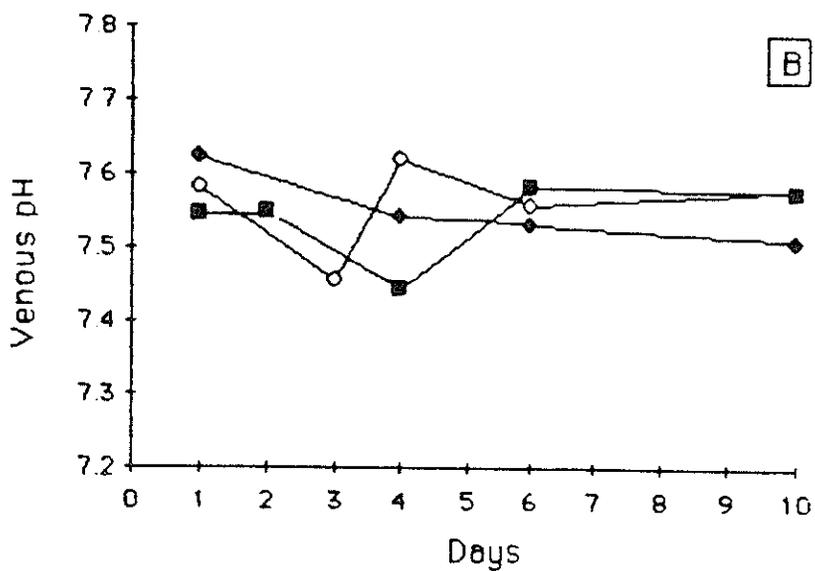
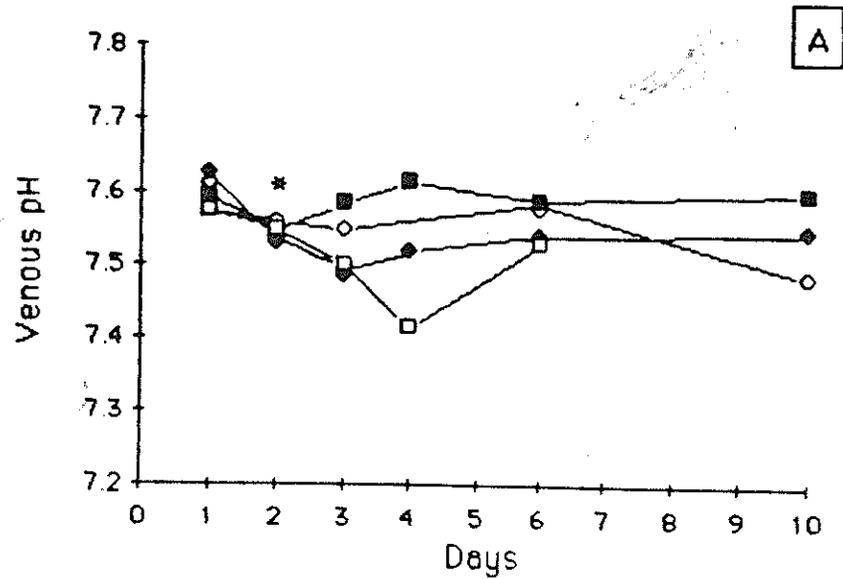


Fig 10 The effect of plastic ingestion (day 1, A) and starvation (B) in the loggerhead sea turtle venous pH.

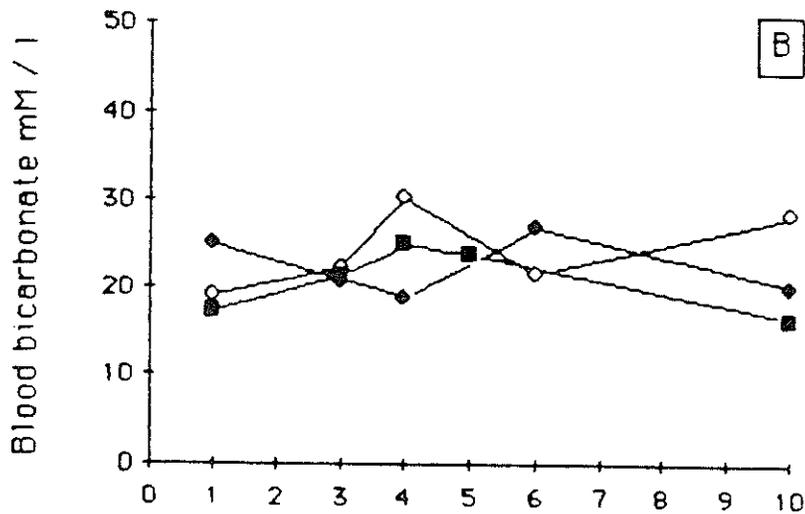
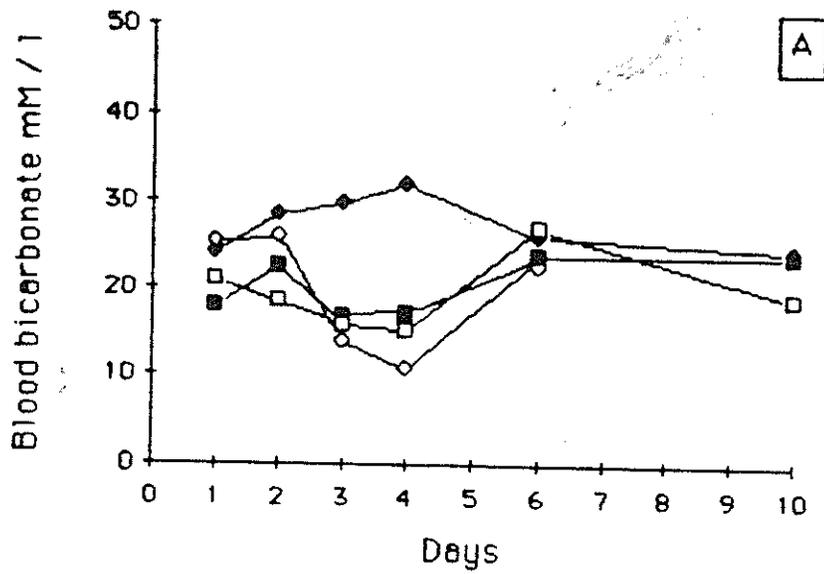


Fig 11 The effect of plastic ingestion (day 1, A) and starvation (B) in the loggerhead sea turtle

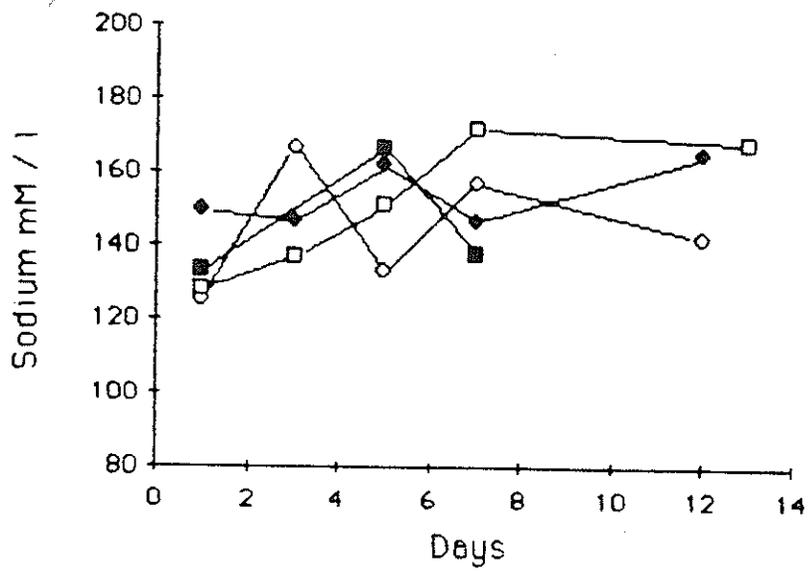


Fig 12 The effect of plastic ingestion (day 1) on plasma sodium concentrations in the green sea turtle

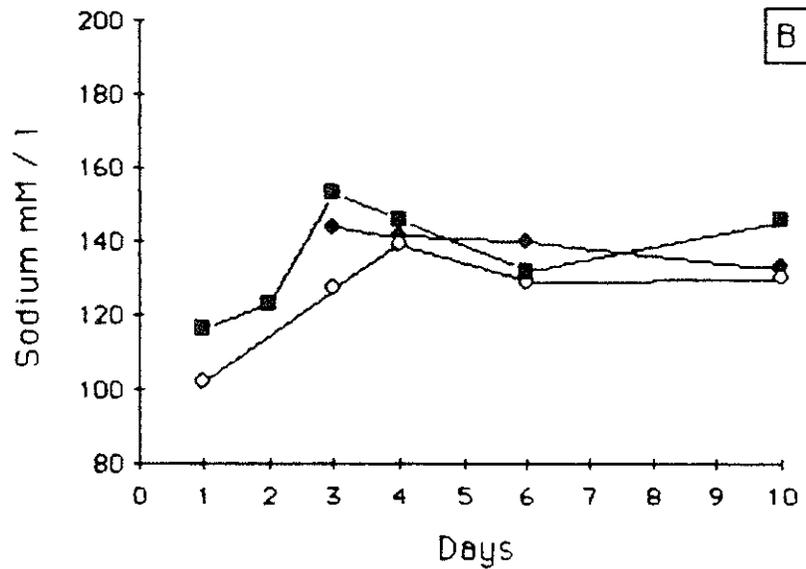
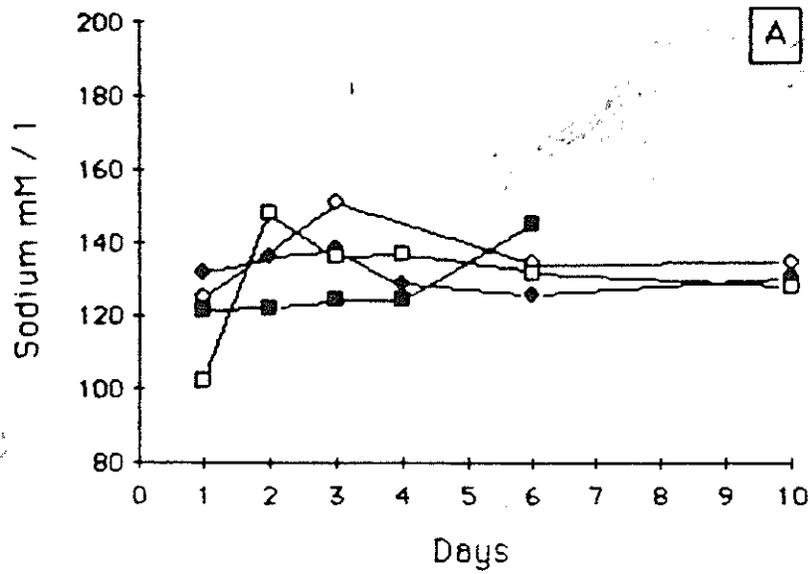


Fig 13 The effect of plastic ingestion (day 1, A) and starvation (B) on plasma sodium concentrations in the loggerhead sea turtle

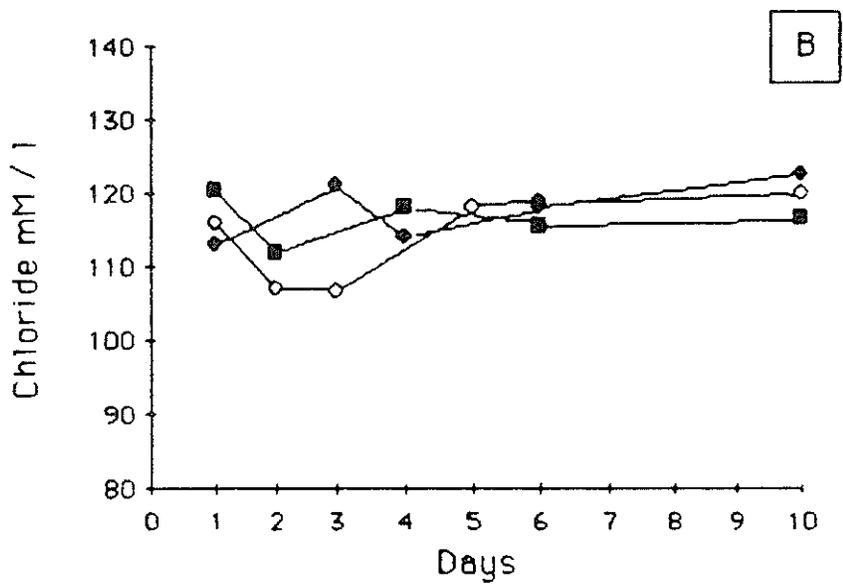
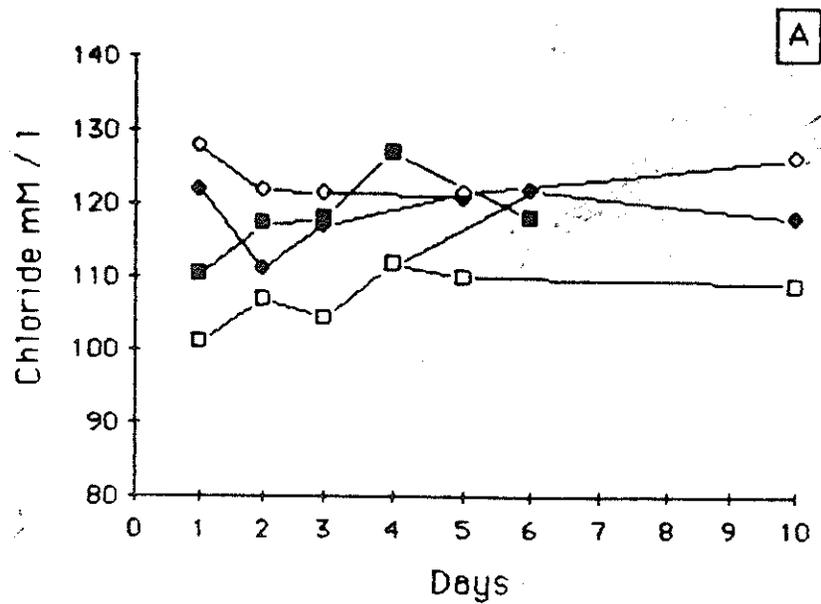


Fig 14 Plasma chloride values following plastic ingestion (day 1, A) and during starvation (B) in the loggerhead sea turtle

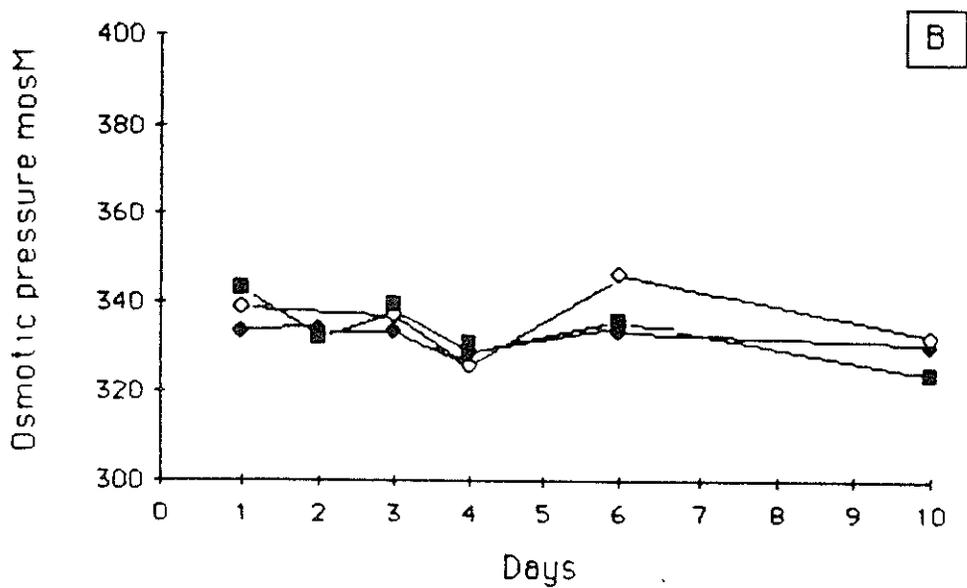
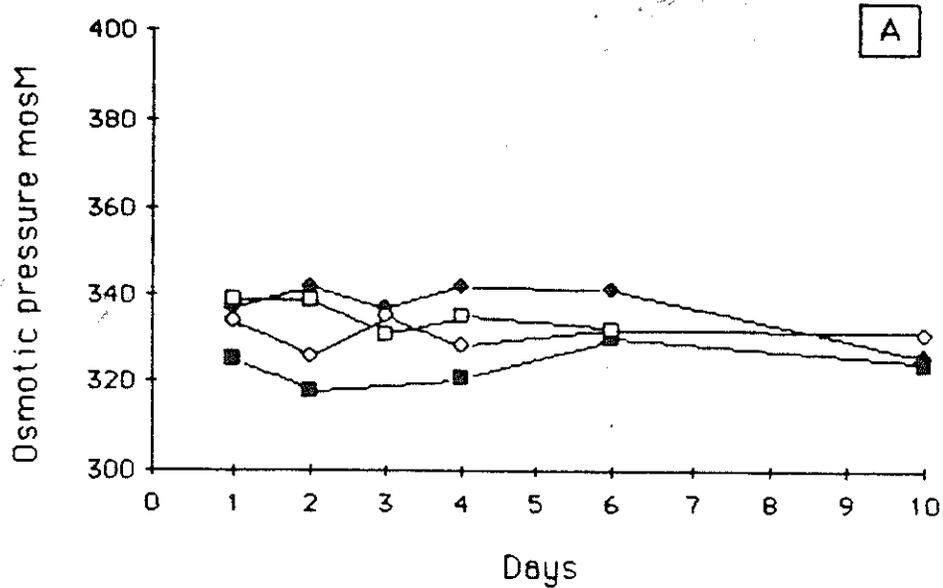


Fig 15 The effect of plastic ingestion (day 1, A) and starvation (B) on the osmotic pressure of loggerhead plasma

Dunbar -Cooper 1987).

Glucose -

In the loggerheads fed plastic blood glucose levels declined for 10 days after feeding (fig 16) but had recovered to initial values by day 14, about the time plastic is expelled from the gut (see below). A least squares linear regression of the relationship between blood glucose (G) and days after plastic ingestion (T) produced the following equation illustrated in fig 16 -

$$G \text{ (mM)} = 6.683 - 0.445 T \quad r=0.866, n=12$$

The average rate of decline in blood glucose was therefore 0.45 mM / day. Interestingly starvation by itself caused a marked fall in blood glucose levels (fig 17 a and b). In both the loggerheads and greens blood glucose levels declined sharply on the second day of starvation at much greater rates than the fed loggerheads who had consumed plastic viz. 2.52mM / day in the green and 2.42 mM / day in the loggerhead.

GTP -

The initial concentration of loggerhead plasma glutamic transaminase (GTP) was 1.67 ± 0.608 , $n=7$, internat. units / ml. GTP values varied somewhat in both the control turtles and the plastic fed turtles for the first three days of the experiment (fig 18 a and b). But after the fourth day in both groups there was a marked decline in values possibly related to the fall in plasma glucose.

Cortisol -

In all samples tested the blood cortisol levels were extremely low ($\leq 1.0 \mu\text{g/dl}$), indicating that the turtles were not stressed by the experimental protocol. Blood cortisol levels have been seen to increase in stressed loggerheads from similar low initial levels (1-3 $\mu\text{g/dl}$ to as high as 37 $\mu\text{g/dl}$ (D. Owens , pers comm.).

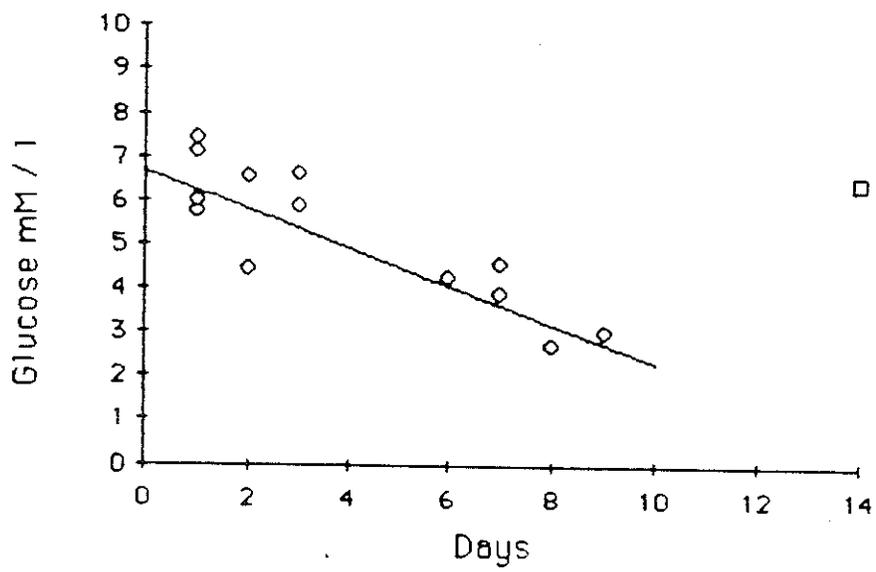


Fig 16 The effect of plastic ingestion on blood glucose levels in the loggerhead sea turtle

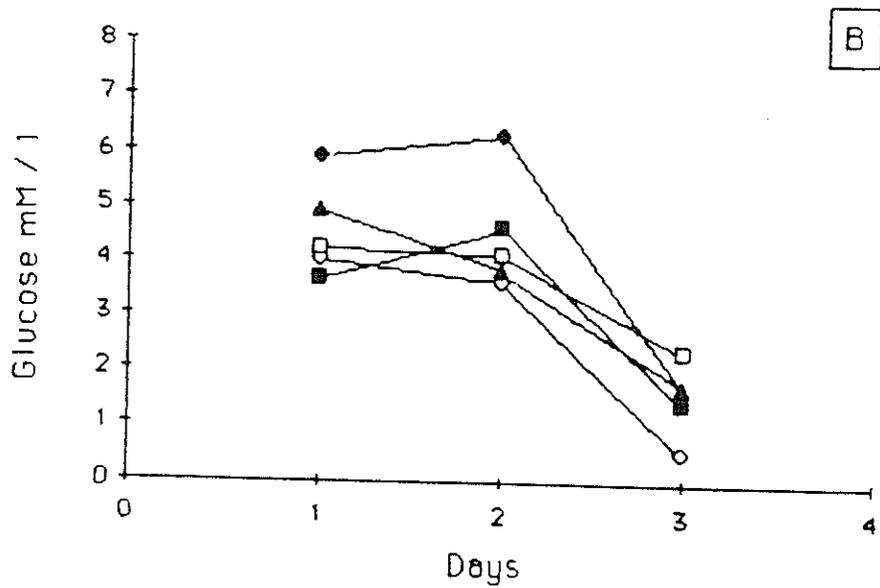
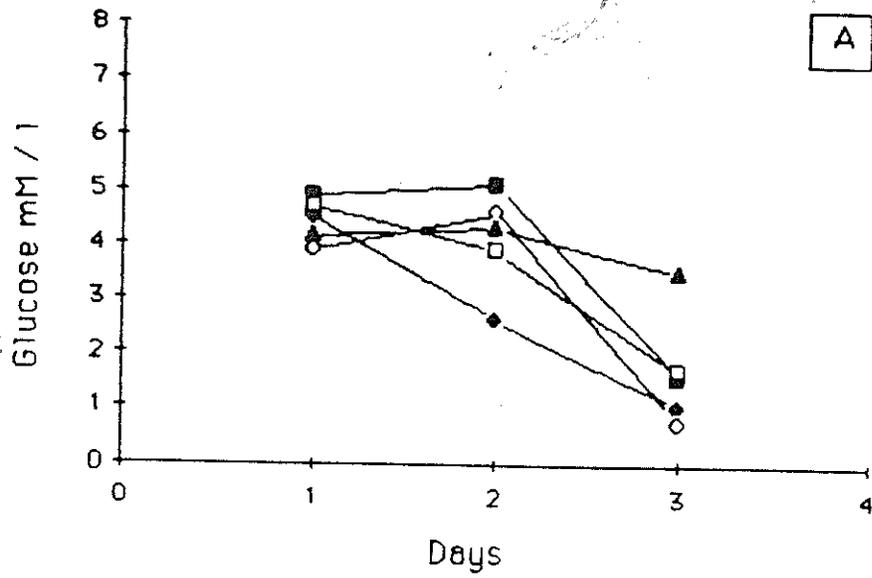


Fig17 The effect of starvation on blood glucose concentrations in the loggerhead (A) and green (B) sea turtles

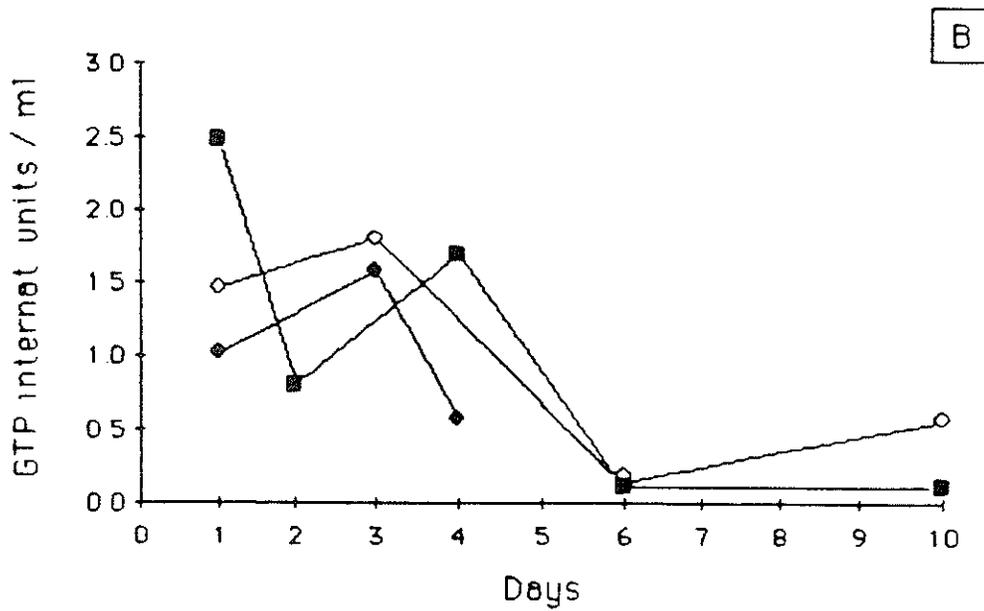
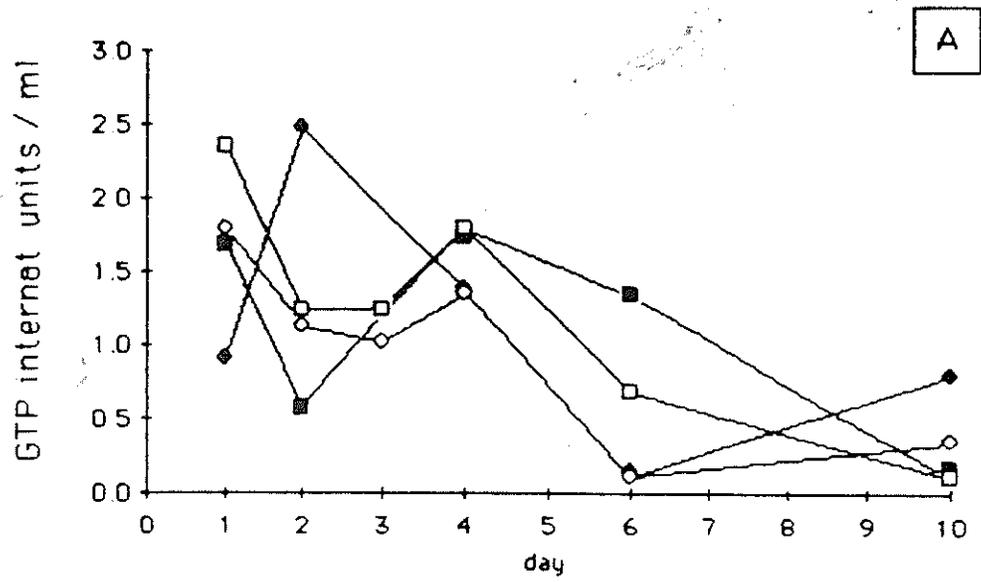


Fig 1B The effect of plastic ingestion (day 1 , A) and starvation (B) on GTP levels in loggerhead plasma

Hematocrit

The hematocrit values did not change over the course of the experiments in either the loggerheads or the greens (Figs 19 a and b). The loggerhead mean value (30%) is slightly less than that found for loggerheads sampled in the wild (35.5%, Lutz and Dunbar -Cooper 1987) and less than that found for the greens (33%) and the latter difference is significant($p < 0.01$).

White blood cells

In the loggerheads the white blood cells initially made up about 0.2% of the whole blood and with one exception the values were reasonably constant ranging between 0.2 and 0.4 % for 10 days after plastic ingestion (fig 20 a). Interestingly the initial values in the green were very much higher the (1.02 %) and showed a greater variation (fig 20b).

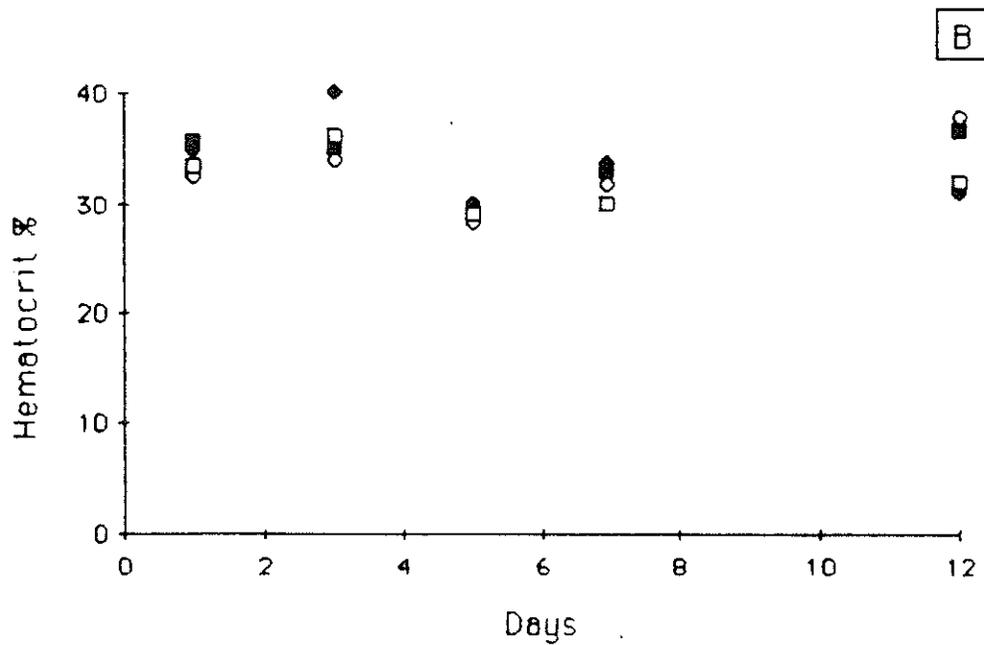
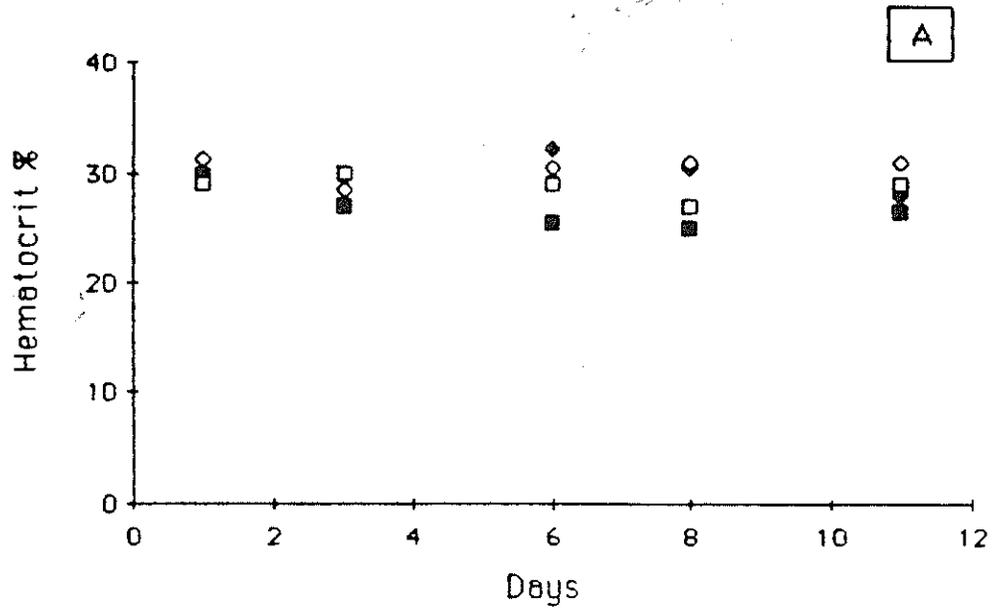


Fig 19 Hematocrits following plastic ingestion in loggerhead (A) and green (B) sea turtles

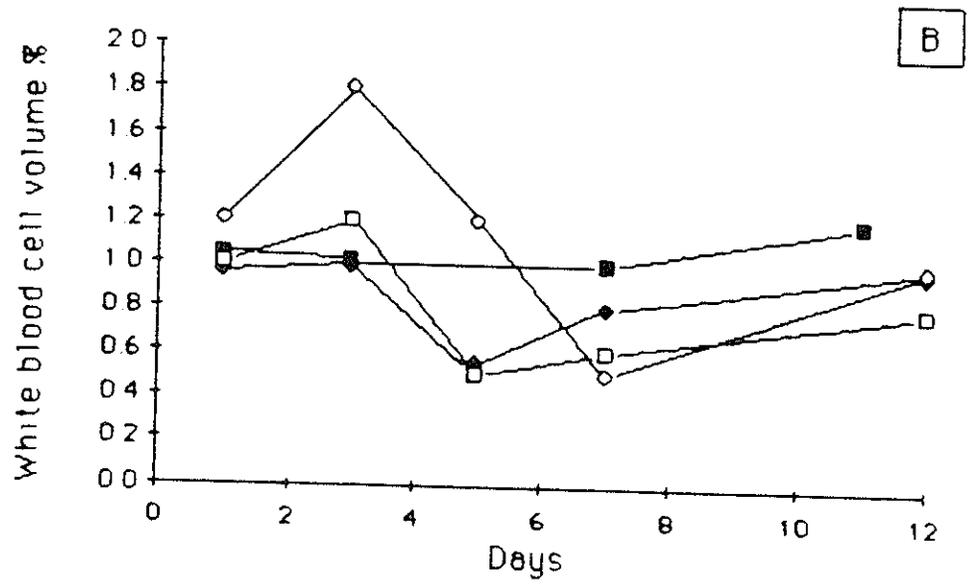
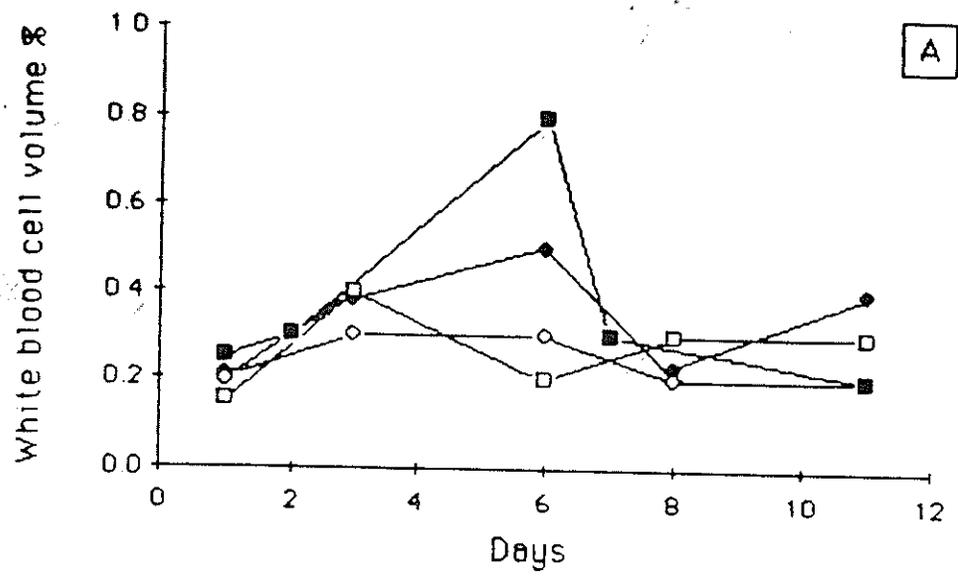


Fig 20 The effect of plastic ingestion (day 1) on white blood cell volume in the loggerhead (A) and the green (B) sea turtle

DISCUSSION

There is now abundant field evidence that sea turtles ingest man-made non-biodegradable debris, particularly plastic sheeting, and most authorities regard this unnatural consumption as source of harm (see lit. rev). The purpose of this study was to document the mode of plastic ingestion in sea turtles and to give a first estimate of how serious the resultant harm might be.

We have been able to demonstrate that both green and loggerhead sea turtles do not discriminate against plastic sheeting when they ingest food intermingled with plastic and, more importantly, they will on occasion, actively seek out and consume plastic. Probable reasons for ingestion are that sea turtles, particularly juveniles, may consume anything that is floating and small enough to swallow (Hughes 1974), and plastic sheets may also be mistaken for natural jelly fish prey (Balazs 1985).

No clear evidence of ill effects from plastic ingestion was found in this set of experiments though it should be noted that the turtles were only allowed to consume small amounts of plastic. In fact, the constancy of many of the physiological parameters over the two weeks of monitoring is evidence that the experimental set up was not, by itself, a perturbing influence.

Further evidence of a lack of stress in both sets, the control group and the group that had eaten plastic, is seen in their low blood cortisol levels. The values are similar to those reported for resting blood cortisol levels for vertebrates in general which are around 1 to 5 $\mu\text{g}/100\text{ml}$ (rainbow trout, 3.8 $\mu\text{g}/100\text{ml}$, Donaldson 1981; loggerhead, 1-3 μg

100/ml, Owens pers com.; dog, 1-5 μ g/100ml, Fraser 1986). The activation of the pituitary interrenal axis is one of the first emergency responses to a wide variety of stress conditions including disease, handling, hypoxia and exposure to pollution. As a result there is a surge in blood corticosteroids often within hours of the stress that will persist during the stress and sometimes for days afterwards (Fraser 1986). Compared to resting values the expected increases in blood cortisol concentrations under stressful conditions can be substantial (16 μ g/100ml in the stressed rainbow trout, Donaldson 1981; app 20 to 38 g/100 ml in the stressed loggerhead, Owens pers comm.).

There was no evidence of plastic ingestion affecting feeding and the handling of food. The rate of food consumption did not change after eating plastic in either the loggerheads or the greens and the average daily consumption was similar for both species (9.79 g/kg/day, green; 7.37 g/kg/day, loggerhead). Wood and Wood (1981) found a similar food intake for green turtles fed pellets (8 to 12 g/kg/day). The food consumption rates found in this study are equivalent to a calorific intake of 44.2 kcal/kg/day for the green and 33.3 kcal/kg/day for the loggerhead.

The gut passage time was unaltered as was the efficiency of food adsorption or the calorific value of the feces. The bacterial composition of the gut was not changed. There was no evidence of blood in the faeces pointing to an absence of mechanical damage as plastic passed through the gut.

No effect of plastic ingestion was detected with respect respiratory behaviour (dive time) or any other of the measured parameters that are directly associated with respiratory physiology viz. metabolic

rate , blood oxygen and carbon dioxide levels, blood acid base status. In fact a very tight relationship was found between blood pCO₂ and bicarbonate concentrations taking all of the loggerheads as a single group (fig 21). The regression equation was

$$pCO_2 \text{ (torr)} = 0.913 \text{ HCO}_3 \text{ (mM)} - 0.028 \quad r=0.918, n=37$$

Blood bicarbonate was also significantly related to pH (p <0.05) although the coefficient of correlation was much lower

$$\text{HCO}_3 \text{ (mM)} = 7.473 + 0.003 \text{ pH} \quad r=0.421, n=37$$

No relationship was found between blood pH and pCO₃ (r=0.044, n=33).

The other blood parameters measured all pointed to healthy animals . Plasma sodium , chloride and osmotic pressure values were steady in all measured turtles and the loggerhead values were very similar to those found for animals in the wild (Lutz and Dunbar- Cooper 1987). There is no evidence therefore for any upset in osmoregulatory abilities. The hematocrit was remarkably constant, an indicator of health, and no marked changes were seen in the proportion of white blood cells . A very substantial increase in white blood cell numbers (400%) was one of the most notable features of sea turtles affected by oil pollution (Vargo et al 1986). No evidence of liver malfunctioning was seen in the lack of increase in plasma glutamic pyruvic transaminase (Fraser 1986).

The rates of change in blood glucose are a possible exception to this pattern . The key observation was that blood glucose declined rapidly in loggerheads that were starved and also fell, although at a lesser rate, in turtles that had been fed plastic sheets. The implication is, therefore , that blood glucose levels in sea turtles are especially sensitive to nutrient uptake from the gut and that this process had been interfered

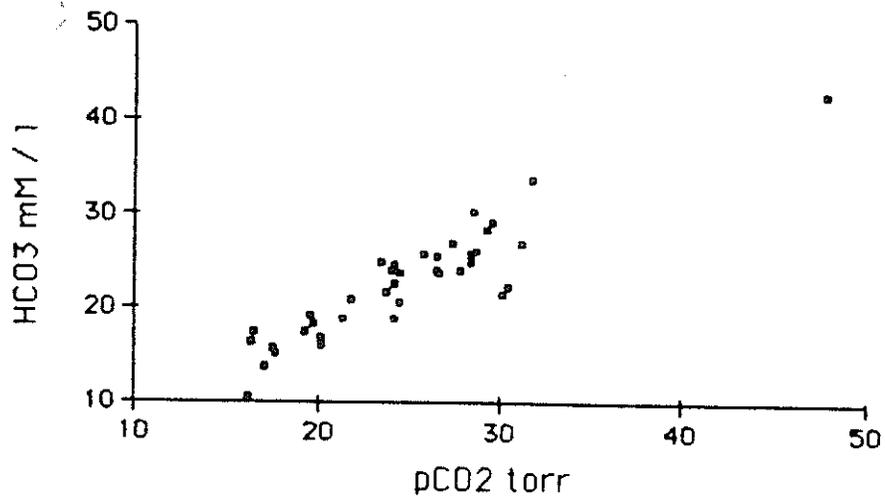


Fig 21 Relationship between venous bicarbonate concentrations and pCO2 in the loggerhead sea turtle.

with in those animals that had consumed plastic. Interestingly the blood glucose concentrations for the control fed loggerheads in this study ($5.23\text{mM} \pm 1.279\text{ mM}$, $n = 10$) were much higher than those recorded in the wild from loggerheads sampled in the Port Canaveral ship channel (approx 1mM , Lutz and Dunbar -Cooper 1987) evidence perhaps that the Canaveral turtles had not been feeding. Blood glucose levels therefore may serve as a sensitive index of nutritional status for turtles both in the laboratory and in the wild.

We can calculate the calorific value of the glucose fall *per se*. For the starving turtles the weight specific rate of decline in glucose is equivalent to $0.18\text{mM} / \text{kg} / \text{day}$. If the extracellular fluid occupies 19% of the loggerhead body volume (Thorson 1968) then per kg animal this body pool is loosing 0.034mM glucose per day, equivalent to about 23 cal in total. This is a trivial amount of the daily heat production estimated from oxygen consumption of 7.15 kcal/g day ($62 \times 24 \times 4.8$) suggesting that the extracellular glucose pool does not serve as a energy pool rather that the lower blood glucose reflects a changed relationship in the rates of glucose release from the liver and consumption by other tissues.

The study did point to some interesting differences in the physiology of greens and loggerheads. On average the greens had a higher hematocrit than the loggerheads (33.3% green, 28.6 % loggerhead) and a higher proportion of white blood cells (0.2% loggerhead, 1.02% green). In the greens the average daily food consumption of the pelleted food was about 32% higher in the green, on the other hand this was offset somewhat by greens having a higher faeces energy content (24 % higher in the green) and therefore a lower efficiency in extracting energy from the food.

SUMMARY

Green and loggerhead sea turtles over the weight range 1 to 16 kg were offered and consumed plastic sheeting. In most instances the ingestion of plastic appeared to occur coincidentally with general feeding and may be accidental or the result of indiscriminate selection but in several occasions we observed both greens and loggerheads to actively seek out and swallow plastic sheets.

At the low levels of ingestion in this study (a single exposure of 1 to 7 pieces of sheeting , 10x10 cms) however, we could detect no significant changes in the features that we measured including : food consumption rates , gut passage times, food adsorption, energy adsorption, dive time , oxygen consumption , blood gases and acid base balance, blood GTP levels , blood cortisol levels (an index of stress), blood ions and osmotic pressure , hematocrit and white blood cell volume. There was however a fall in blood glucose levels, similar to , but at a much slower rate , than that produced by starvation.

We can conclude that sea turtles can actively seek out and consume plastic sheeting but that, except for a possible minor change in glucose metabolism , the effects appeared to be innocuous at the levels of ingestion we allowed in these experiments.

We can say nothing , however about what might happen at higher levels of ingestion and it would be most unfortunate if our study was used to support the position that the ingestion of non biodegradable debris at a level less than gut strangulation is without consequence to sea turtles. It is very possible that an increased burden will cause harm since in

vertebrates in general a partial blockage of the gut can cause an interference with nutrition, loss of electrolytes and blood in the faeces. Very much higher levels of plastics than we used in this study have been found in nature including large pieces of plastic bags twisted throughout the intestine, and there is evidence of repeated ingestion in some specimens (Balazs 1985). Clearly further work is called for to examine the effects of higher and more realistic levels of plastic ingestion. This study will serve as a justification and as the scientific base for such work.

LITERATURE CITED

- Balazs, G.H. (1985). Impact of ocean debris on marine turtles: Entanglement and ingestion. In: Proceedings of the Workshop on the Fate and Impact of Marine Debris. R.S. Shomura and H.O. Yoshida (ed.). U.S. Dep. Commer., NOAA Tech. Memo. NMFS, NOAA-TM-NMFS-SWFC-54.
- Bentley, T.B., and A. Dunbar-Cooper. (1980). A blood sampling technique for sea turtles. Contract No. NA-80-GE-A-00082 for the National Marine Fisheries Service. September.
- Bjorndal, K.A. (1985). Use of ash as an indigestible dietary marker. *Bull. Mar. Sci.* 36(1):224-230.
- Brongersma, L.D. (1969). Miscellaneous notes on turtles, IIA-B. *Kon. Ned. Akad. Wetensch., Proc. Ser. C. Biol. Med. Sci.* 72:76-102.
- Cawthorn, M.W. (1985). Entanglement in, and ingestion of, plastic litter by marine mammals, sharks, and turtles in New Zealand waters. In: Proceedings of the Workshop on the Fate and Impact of Marine Debris. R.S. Dhomura and H.O. Yoshida (eds.). NOAA Tech. Memo. NMFS, NOAA-TM-NMFS-SWFC-54.
- Conover, R.J. (1966). Assimilation of organic matter by zooplankton. *Limnol. Oceanogr.* 11:338-345.
- Cornelius, S.E. (1975). Marine turtle mortalities along the Pacific coast of Costa Rica. *Copeia.* 1975:186-187.
- Den Hartog, J.C., and M.M. Van Nierop. (1984). A study on the gut contents of 6 leathery turtles from British waters and from the Netherlands. Zool. verh (Leiden). 1-36.
- Donaldson, E.M. (1981). The pituitary - inter renal axis as an indicator of stress in fish. In Stress and Fish. (ed.) A.D. Pickering. pp. 12-47. Academic Press. London.

- Duguy, R. (1983). La tortue luth (Dermochelys coriacea) sur les cotes de France. Ann. Soc. Sci. Nat. Charente-Marit. Suppl., 38 p.
- Duguy, R, and M. Duron. (1981). Observations of leatherback turtles dermochelys-coriacea on the coasts of France. Ann. Soc. Sci. Nat. Charente-Marit. 6(9):1015-1020.
- Duguy, R., and M. Duron. (1982). Observations de tortues luth sur les cotes de France en 1981. Ann. Soc. Sci. Nat. Charente-Marit. 6(9):1015-1020.
- Fraser, C.M. (ed.). (1986). The Merk Veterinary Manual. 6th ed. Merk and Co. Rahway, N.J.
- Fritts, T.H. (1982). Plastic bags in the intestinal tracts of leatherback marine turtles Eromochelys-Coriacea. Herpetol. Rev. 13(3):72-73.
- Hays de Brown, C. and W.M. Brown. (1981). The status of sea turtles in the Southeastern Pacific: Emphasis on Peru. In: Bjorndal, K. (ed.). Proceedings of the World Conference on Sea Turtle Conservation. (In Press).
- Henry, R.J. (1974). Clinical chemistry: Principle and techniques. 2nd Ed. Harper and Row, New York.
- Horsman, P.V. (1982). The amount of garbage pollution from merchant ships. Mar. Pollut. Bull. 13:167-169.
- Hughes, G.R. (1974). The sea turtles of South-East Africa. I. Status, morphology and distribution. S. Afr. Assoc. Mar. Biol. Res., Oceanogr. Res. Inst. Invest. Rep. 35, 144p.
- Kraus, D.R., and D.C. Jackson. (1980). Temperature effects on ventilation and acid-base balance of the green turtle. Am. J. Physiol. 239:254-258.
- Lutz, P.L., and A. Dunbar-Cooper. (1987). Variations in the blood chemistry of the loggerhead sea turtle, Caretta caretta. Fish. Bull. Vol. 85, No. 1.
- Lutz, P.L., and T.B. Bentley. (1985). Adaptions for diving in the sea turtle. Copeia. 1985:671-697.

- Lutz, P.L., and A. Dunbar-Cooper. (1987). Variations in the blood chemistry of the loggerhead sea turtle Caretta caretta. Fishery Bulletin: 85:37-44.
- Mortimer, J.A. (1981). The feeding ecology of the West Caribbean green turtle (Chelonia mydas) in Nicaragua. Biotropica 13(1):49-58.
- Mrosovsky, N. (1981). Plastic jellyfish. Mar. Turtle Newsl. 17:5-7.
- Newman, M.W., P.L. Lutz, and S.C. Snedaker. (1982). Temperature effects on feed ingestion and assimilation efficiency of nutrient by the Malasian prawn, Macrobrachium rosenbergii. J. World. Marine Soc. 13:95-103.
- Osterburg, C. (1986). Waste disposal - where should it be? Land or Sea? 6th Int. Ocean. Disp. Symp. 181.
- Prange, H.D., and P.L. Lutz. (1980). Responses of sea turtles to graded hypoxia. The Physiologist, 23(4):71.
- Severinghaus, J.W. (1965). Blood gas concentration. In Handbook of Physiology. Respiration, pp. 1475-1487. Washington: Am. Physiol. Soc.
- Thorson, T.B. (1968). Body fluid partitioning in reptilia. Copeia. 1968 3:592-601.
- Van Dam, L. (1984). Mat of artificial seaweed helps reverse beach erosion. St. Petersburg Times, 9 April 1984, p. 21.
- Van Dolah, R.F., V.G. Burrell, Jr., and S.B. West. (1980). The distribution of pelagic tars and plastics in the South Atlantic Bight. Mar. Pollut. Bull. 11:352-356.
- Van Franeker, J.A. (1985). Plastic ingestion in the North Atlantic fulmar. Marine Pollution Bulletin, Vol. No. 9, pp. 367-369.
- Van Nierop, M.M. and J.C. den Hartog. (1984). A study on the gut contents of five juvenile loggerhead turtles, Caretta caretta (Linnaeus) (Reptilia, Cheloniidae), from the south-eastern part of the North Atlantic Ocean, with emphasis on coelenterate identification. Zool. Meded. (Leiden) 59(4):35-54.

Vargo, S., P.L. Lutz, D. Odell, E. van Vleet, and G. Bossart. (1986). Effects of oil on sea turtles. Final report to M.M.S., U.S. Department of the Interior. Report No. 14-12-0001-30063.

Wood, F.E., and J.R. Wood. (1981). Growth and Digestibility for the green turtle (Chelonia mydas) fed diets containing varying protein levels. Aquaculture. 25:269-274.