

Behaviour of juvenile green turtles (*Chelonia mydas*) before and after fibropapillomatosis tumour removal

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Introduction

Wild sea turtle populations worldwide, including in Florida, are under increasing threat from a fatal virulent tumour disease, fibropapillomatosis (FP) (Herbst 1994; Duffy *et al.* 2018; Farrell *et al.* 2018; Duffy & Martindale 2019; Jones *et al.* 2016), potentially undermining turtle conservation efforts (Foley *et al.* 2005; Jones *et al.* 2016). Green sea turtles (*Chelonia mydas*) are the species most affected by FP (Fig. 1), but it has also been detected in all other sea turtle species (Herbst 1994). While FP tumours are generally not malignant, their detrimental consequences can result in susceptibility to other risks, including predation, parasitism and secondary diseases and infections (Vittecoq *et al.* 2015). Tumours can be large enough to restrict feeding, swimming, and reproductive abilities, and cause immunosuppression (Aguirre & Lutz 2004; Herbst 1994; Jones *et al.* 2016; Williams Jr *et al.* 1994). Currently, surgery is the primary treatment for turtles with FP, but at least 40% of turtles that have tumours surgically removed experience regrowth (Page-Karjian *et al.* 2012; Duffy *et al.* 2018; Farrell *et al.* 2018). Fibropapillomatosis is now spreading to areas where it has not previously been observed (Duarte *et al.* 2012; Foley *et al.* 2007; Page-Karjian *et al.* 2014; Hirama & Ehrhart 2007; Hargrove *et al.* 2016; Foley *et al.* 2015), which means that the number of stranded turtles requiring treatment for FP is also increasing.

There are still many gaps in our understanding of sea turtles in general, with ongoing discussion about the most effective conservation strategies (Hamann *et al.* 2010). Research on the behaviour of turtles in the wild tends to focus more broadly on foraging and migration habits (Dodge *et al.* 2014; Hochscheid 2014; Heithaus *et al.* 2002; Godley *et al.* 2002). One study made a comparison between the behaviour of tumour-bearing and non-tumour-



Fig. 1. Green turtles (*C. mydas*) at the UF Whitney Laboratory Sea Turtle Hospital, Florida, afflicted with fibropapillomatosis tumours. Top left: juvenile green turtle being evaluated prior to surgery. Top right: fibropapillomatosis tumour after surgical excision from patient. Bottom: green sea turtle in hospital rehabilitation tank.

bearing wild turtles (Brill *et al.* 1995), and found that tumour-bearing turtles surfaced less frequently at night than those without tumours, suggesting that FP tumours may impact turtle respiration. Compromised respiration therefore may affect their resting and foraging behaviour, negatively impacting their energy budgets (Brill *et al.* 1995). Some research has been carried out into the behaviour of healthy captive green turtles (Therrien *et al.* 2007), but to our knowledge the behaviour of turtles being treated for FP has not been documented. In other reptile species, minimally invasive procedures such as microchipping have been found to have a prolonged effect on stress levels (Langkilde & Shine 2006). Given that surgical tumour removal is more invasive than microchipping, it may be assumed that surgery would also have an effect on sea turtle stress levels. Although it is broadly accepted that FP

tumour removal can increase the probability of sea turtle survival (Herbst *et al.* 1995; Jones *et al.* 2016; Work *et al.* 2004), it is not known exactly how surgical removal of FP tumours from sea turtles affects their behaviour, and no formal studies have been carried out to determine the optimal length of time that turtles should be held when tumours have been removed prior to being returned to the wild (Divers & Mader 2005). While turtles that have received surgery for injuries such as embedded fishing hooks may be released relatively quickly (Barreiros 2015), turtles that have a heavy FP tumour load can be held for a longer period of time, possibly up to a year, to ensure that regrowth does not occur (Divers & Mader 2005), providing an opportunity to observe turtle behaviour after surgery in greater detail than is possible in the wild.

Here we present observations and comparisons of the behaviour of nine juvenile green sea turtles (*C. mydas*) held at the University of Florida's Whitney Laboratory for Marine Bioscience and Sea Turtle Hospital. Observations were made before they had FP tumours surgically removed, one week after surgery, and one month after surgery, to determine whether tumour removal directly affects turtle activity budgets, and how turtle behaviour changes as they recover from surgery.

Methods

Turtles

Participating turtles were patients at the University of Florida's Whitney Laboratory for Marine Bioscience and Sea Turtle Hospital, on the north-eastern coast of Florida. Sea turtles that were stranded or injured and afflicted with FP were cared for at the hospital to regain strength until they were fit enough to have FP tumours surgically removed by CO₂ laser resection (Burkhalter & Norton 2019). In many cases, the tumours visibly hampered movement and reduced flexibility and motility of the turtles' flippers (Fig. 1). Nine juvenile green turtles (*C. mydas*) at the Sea Turtle Hospital participated in this study, all of which were suffering from FP (Table 1). They had received any treatment necessary immediately after admission to ensure they were in good physical condition prior to undergoing surgery, and were under regular veterinary observation. The period of recovery time (Table 1) between initial admission and treatment (if any) and time of behaviour observations and surgery meant it was likely that the turtles had recovered from the immediate acute stress of being stranded and transported to the hospital (Gregory *et al.* 1996), and that they were habituated to their surroundings and the tank prior to observations being carried out.

Turtles were held in circular tanks of 240 cm diameter, holding 2,270 litres of continuously filtrated sea water, which were partitioned in the middle with one turtle on each side of the partition, except for Banana, who was held

Table 1. Carapace length, weight, origin, condition and tumour burden scores of the nine green sea turtles observed during this study on their arrival at the Whitney Laboratory for Marine Bioscience Sea Turtle Hospital, duration from admittance to first surgery and the number of days in care.

ID	Straight carapace length (cm)	Weight (kg)	Origin (Florida)	Condition on arrival	FP burden score (Rossi <i>et al.</i> 2016)	Admittance to surgery time (months)	Days in care (outcome)
Major	40.0	7.6	Daytona Beach	FP, emaciated	Moderate (81.5)	5.5	302 days (released)
Emma	37.8	5.8	Daytona Beach	FP, floating	Mild (26.6)	2.5	148 days (released)
Mean Joe Green	33.7	6.6	Crescent Beach	FP, cold stunned	Mild (0.6)	2	523 days (released)
Banana	64.7	36.3	Banana River	FP, boat strike	Mild (1.6)	5.5	218 days (released)
Sebod	36.6	5.6	Port Orange	FP, floating, boat strike	Mild (1.6)	1.25	93 days (released)
Remi	37.8	3.8	South Daytona	FP, emaciated, lethargic	Severe (>205.6)	2	344 days (released)
Tamatooa	35.1	4.6	St Augustine	FP, floating	Mild (22.1)	0.5	87 days (released)
RT	32.8	4.2	Ponce Inlet	FP, floating, good body condition	Moderate (>66.5)	0.75	150 days (died in care)
Rollie	33.6	4.4	South Daytona	FP, floating, good body condition	Moderate (>86.5)	2	241 days (euthanised)

individually in an un-partitioned tank. Note that although turtles were assigned identifying names, their sex was not known due to the difficulty of sexing juvenile sea turtles. The turtles were housed and provided with veterinary care according to best rehabilitation practice (Manire *et al.* 2017). Neither turtles nor food crossed the partition. Patients were not fed to satiation, rather each individual had a personally tailored diet designed by the veterinary care team, based on factors such as the turtle's age, condition and time in rehabilitation. Therefore, food was not present during all observation periods.

Behaviour

Turtle behaviour was divided into five categories (Table 2), based on preliminary observations and on previously described captive turtle behaviour (Therrien *et al.* 2007).

Table 2. Behaviour categories of captive green turtles (*C. mydas*)

Behaviour	Definition
Resting	Any period of inactivity.
Repetitive swimming	Swimming in repetitive patterns around/back and forth across tank.
Random swimming	Swimming in a non-repetitive way.
Investigating	Moving directly towards and observing or touching object or person.
Grooming	Rubbing carapace or body against flipper or tank.
Feeding	Manipulating, investigating or eating food.

Observations

Observations were carried out under a Florida Fish and Wildlife Conservation Commission (FWC) consent permit (#760) associated with the rehabilitation and research activities of the hospital conducted under FWC permits MTP-16-228 and MTP-16-236, and with ethical approval from the University of Florida Institutional Animal Care and Use Committee (IACUC). Turtles were observed for sampling periods of 20 minutes at intervals spread evenly throughout the day from 10 am to 5 pm, allowing for husbandry and veterinary care requirements throughout the day, and ensuring that behaviour over the course of the day was captured. Because turtles were held in individual tanks, continuous focal sampling was carried out (Altmann 1974). The total time spent performing each type of behaviour (Table 2) during each 20 minute

sampling period was recorded. Five observation samples were obtained for each turtle before surgery, one week after undergoing surgery, and one month after undergoing surgery, providing a total of fifteen samples for each turtle. The five observations of each turtle under each condition (i.e. pre-surgery, one week post-surgery or one month post-surgery) were made as close as possible to each other. Where priority had to be given to husbandry and veterinary care of the turtles or other scheduling constraints, observations were made over the course of more than one day. Observations were carried out by a number of individuals who were trained by the first author to ensure consistency between observers in behaviour recording.

Analysis

Data were analysed using SPSS 24.0 for Windows. Distributions and variances of data for all behaviours for each individual during each period were checked; any datasets not displaying normal distribution were log transformed. The proportion of time per observation session ($n = 5$) that each turtle spent exhibiting each behaviour before they underwent surgery was compared with their behaviour one week after surgery and one month after surgery. Paired t-tests were used to assess changes in individual turtle behaviour, between pre-surgery observations and one week post-surgery, and also between pre-surgery observations and one month post-surgery. Additionally, the proportion of time per observation session ($n = 5$) that all turtles spent exhibiting each behaviour before they underwent surgery was compared (Wilcoxon signed-rank test) with one week after surgery and one month after surgery.

Results

There were some significant behaviour changes observed in turtles after they had undergone surgery. These are illustrated in the activity budgets in Figure 2, and summarised in Table 3 and Figure 3. All turtles exhibited some significant behavioural changes. Generally, patients spent significantly more time resting 1 week after surgery compared with the amount of time spent resting prior to surgery (Figs 2 & 3). On average across the group by one month post-surgery resting had returned to pre-surgery levels (Figs 2 & 3). 'Emma' and 'Rollie' spent significantly reduced periods resting one week post-surgery compared with their pre-surgery resting levels (paired t-test: $p = 0.002$ and $p = 0.0004$, respectively) (Fig. 2). In line with the increase in resting one week post-surgery, both random swimming and repetitive swimming decreased across the group, returning to near pre-surgery levels one month post-surgery (Fig. 3). 'Rollie' and 'Sebod' significantly increased their time random swimming one month post-surgery (paired t-test: $p = 0.0057$ and $p = 0.003$, respectively), while 'Emma' increased and 'RT' decreased repetitive swimming one month post-surgery (paired t-test: $p = 0.0064$, and $p = 0.016$, respectively).

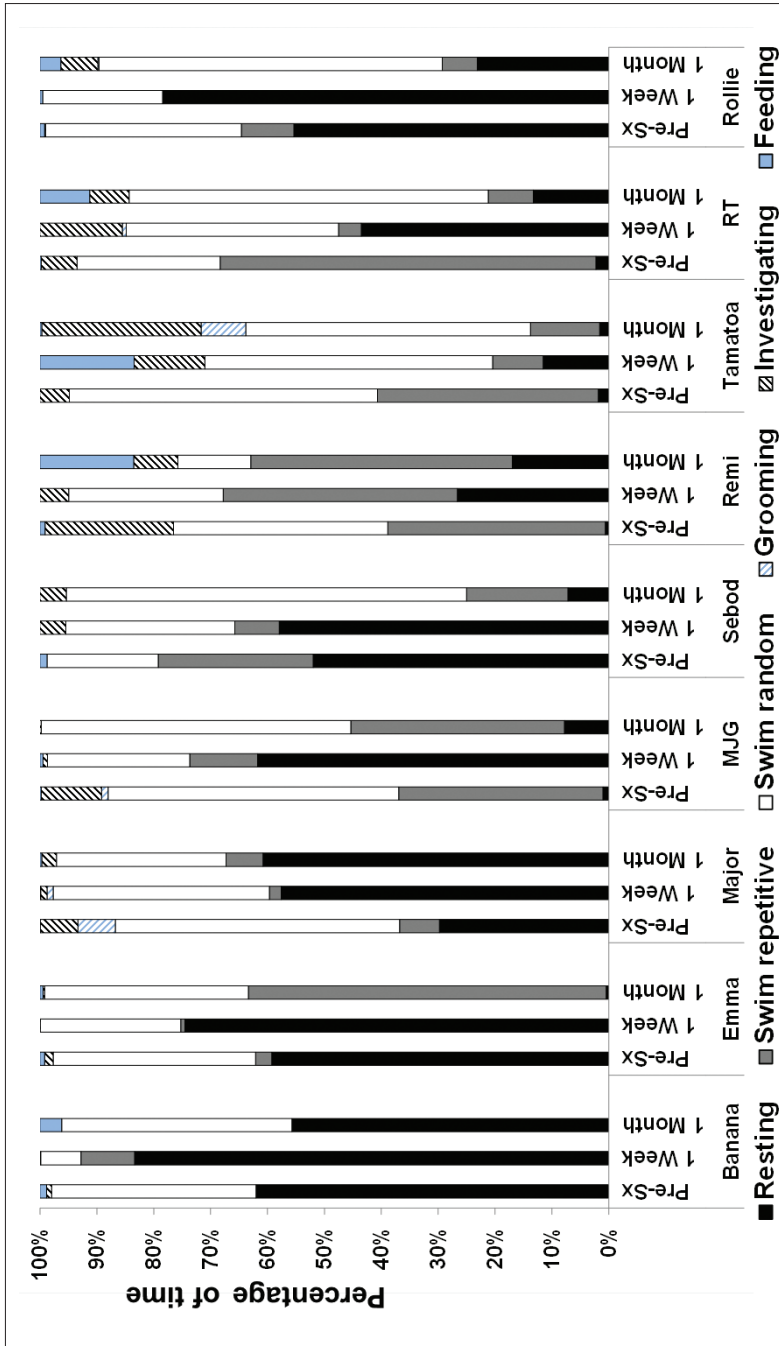


Fig. 2. Average activity budgets of each of nine juvenile green turtles prior to surgical removal of FP tumours (1), one week after surgery (2), and one month after surgery (3). Activity budgets show the mean percentage of time each turtle was observed carrying out each activity. (n = 5). MJG = 'Mean Joe Green'. Pre-Sx = pre-surgery.

Table 3. Statistically significant ($p < 0.05$, paired t-test) differences in behaviour observed in turtles one week after surgery and one month after surgery, compared with their behaviour prior to surgery.

Patient	One week post-surgery	One month post-surgery
Major	Decreased investigating ($p = 0.023$)	
Mean Joe Green	Increased resting ($p = 0.005$) Decreased investigating ($p = 0.039$) Decreased random swimming ($p = 0.0487$) Decreased repetitive swimming ($p = 0.024$)	Decreased investigating ($p = 0.040$)
Emma	Decreased investigating ($p = 0.005$)	Decreased resting ($p = 0.002$) Increased repetitive swimming ($p = 0.006$) Decreased investigating ($p = 0.005$)
Banana	Decreased random swimming ($p = 0.035$)	
Sebod	Increased investigating ($p = 0.042$)	Increased random swimming ($p = 0.003$) Increased investigating ($p = 0.025$)
Remi	Increased resting ($p = 0.001$) Decreased investigating ($p = 0.007$)	
Tamatoa		Increased investigating ($p = 0.005$) Increased grooming ($p = 0.023$)
RT	Increased resting ($p = <0.001$) Decreased repetitive swimming ($p = 0.001$)	Decreased repetitive swimming ($p = 0.016$)
Rollie		Decreased resting ($p = 0.0004$) Increased investigating ($p = 0.0003$) Increased random swimming ($p = 0.0057$)

'Emma' was the only turtle to increase repetitive swimming significantly one month after surgery, compared with pre-surgery levels. 'Emma' spent somewhat more time resting a week after surgery, but one month after surgery the time spent resting had dropped dramatically (paired t-test: $p = 0.002$). There was a corresponding highly significant increase in repetitive swimming one month after surgery (paired t-test: $p = 0.0064$), while the amount of time investigating, already low prior to surgery, decreased significantly after surgery and remained low one month after surgery (paired t-test: $p = 0.005$).

The combined average time that all turtles spent exhibiting each behaviour pre-surgery, one week post-surgery, and one month post-surgery is shown in Figure 3. Behaviours that would be considered positive, including random swimming and investigating, are presented separately to behaviours that could be indicative of increased stress levels or poor health (in this case, prolonged periods of resting and repetitive swimming). Generally, negative behaviours trended lower one month post-surgery compared with pre-surgery levels, while positive behaviours trended higher (Fig. 3). While across the nine turtles the changes in these behaviours were only a trend, in individual turtles many of these desirable changes were statistically significant (Table 3). Across the nine turtles random swimming, resting and repetitive swimming also changed significantly between one week post-surgery and one month post-surgery (Fig. 3).

Discussion

Significant changes in behaviour occurred in all turtles that were monitored for this study. These changes varied between individuals but some noteworthy similarities were observed: resting significantly increased one week after surgery as the turtles were presumably still recovering and longer-term desirable behaviours such as investigating, random swimming and grooming significantly increased one month after surgery (Figs 2 & 3).

In seven of the turtles, behaviours were observed to change, and remained altered one month after surgery: 'RT' decreased repetitive swimming, 'Mean Joe Green' and 'Emma' decreased investigating, whereas 'Sebod' and 'Rollie' increased investigating. 'Tamatoa' increased grooming one month post-surgery.

Many behaviours returned to pre-surgery levels after one month. This suggests that the behavioural changes that were observed occurred in response to the surgery, but not necessarily to the absence of FP tumours, although the general trend towards more positive changes one month post-surgery (which were significant in some individuals) could possibly be influenced by the absence of FP tumours.

While an increase in resting (on average there was twice as much resting one week post-surgery) is unsurprising during the period immediately after

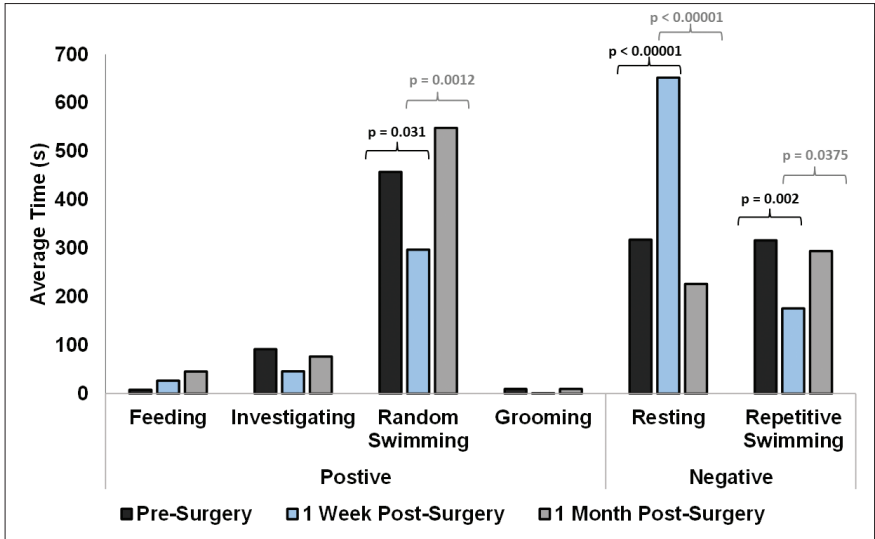


Fig. 3. Average time (seconds) spent by all turtles exhibiting each behaviour pre-surgery, one week post-surgery and one month post-surgery. Behaviours have been categorised as 'positive' or 'negative'. (Nine turtles; per turtle observation N = 5; observation time = 20 minutes). All statistically significant changes have been indicated using a chain bracket with the p-value above (Wilcoxon signed-rank test).

surgery, other changes in behaviour may also be indicative of both physical and psychological statuses of the animals (Mills *et al.* 2014). There has been some research into the physiological effects of FP tumours (Aguirre *et al.* 1995), but less is known about the immediate behavioural effects of tumour removal. Stereotypic behaviour such as repetitive swimming patterns, which were observed to significantly increase in 'Emma', may be indicative of increased stress levels, induced either by the stress of surgery or the extended period of captivity. Decreased amounts of time spent investigating, also observed in four turtles, may also be indicative of increased stress levels; exploratory behaviour is considered to be a positive behaviour in captive sea turtles and other reptiles (Burghardt *et al.* 1996; Therrien *et al.* 2007). In contrast, four turtles increased the amount of time spent investigating, swimming randomly and/or grooming, suggesting that the removal of FP tumours had a positive behavioural effect on these individuals after the acute stress of surgery had subsided.

There were several changes in individual activity budgets that merit discussion and further investigation. The near-significant prolonged period of increased resting observed in 'Major' (paired t-tests: one week after surgery: $p = 0.0797$; one month after surgery: $p = 0.0672$) indicate that this individual in particular may have been undergoing a longer recovery

period after the surgery than some of the other turtles. Changes in 'Emma's behaviour suggested increased stress levels over a prolonged period. For such turtles it would be worth investigating whether behavioural enrichment devices could reduce stress levels. The more positive behavioural changes of 'Sebod', 'Tamatoa', 'Rollie' and 'RT' following surgery suggest that the behavioural effects of removing FP tumours may vary for each individual and the time-scale of these effects may also vary. The general increase in locomotive behaviours (such as investigating and random swimming) one month after surgery could be a direct result of the turtles no longer being encumbered by FP tumours. Variations in the tumour burden of each turtle means that FP tumours affect each turtle differently, as does their removal. An improved understanding of the behavioural effects of surgery could help inform post-operative husbandry practices, including provision of enrichment and consideration of each turtle's particular needs.

Studies of physical characteristics in turtles suffering from FP have been previously carried out (Adnyana *et al.* 1997; Aguirre *et al.* 1995), but this study is, to our knowledge, the first to examine behavioural effects of FP tumour removal. Because there is a great deal of variation in sea turtle behaviour both at baseline and after they have been through surgical treatment for FP, larger-scale and longer-term studies that correlate tumour burden with behaviour patterns before and after surgery would provide a clearer picture of the behavioural effects of tumour removal. It is recommended that future studies carry out periodic follow-up observations of post-operative turtles until such time that they are deemed by the veterinary and husbandry team to be fit to be released back to the wild, thereby providing greater clarity into the short- to medium-term effects of FP tumour removal. This will add greatly to our current understanding of the effect of cancer on endangered wildlife (Whilde *et al.* 2017), contribute to quantifying the results of treatments (Duffy & Martindale 2019), and optimise the psychological and physiological wellbeing of wild sea turtles while in a hospital or rehabilitation setting.

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