Stereotypic pacing and faecal corticosterone metabolites as non-invasive indicators of stress in rehabilitating green turtles (*Chelonia mydas*)

Melanie C. Smith[1]*, Christopher J. Michaels[2] and Edward J. Narayan[3]*

[1] Institute of Zoology, Outer Circle, Regent's Park, London NW1 4RY, UK

[2] ZSL London Zoo, Outer Circle, Regent's Park, London NW1 4RY, UK

[3] School of Agriculture and Food Sciences, Faculty of Science, The University of Queensland, St Lucia, QLD 4072, Australia

*Corresponding authors:

Melanie C. Smith, email: mcsmith7@rvc.ac.uk

Edward Narayan, email: e.narayan@uq.edu.au

Introduction

All species of marine turtle are threatened with extinction (six species) or listed as Data Deficient (one species) (IUCN 2018). Many populations are in global decline and face threats such as bycatch, pollution, climate change, egg predation, emerging infectious diseases, loss of important habitats such as nesting beaches, and other environmental anthropogenic hazards (Gall & Thompson 2015; Santos et al. 2015; Varghese 2015; Rees et al. 2016; Lutcavage et al. 1997). In Australia, a species recovery plan (Commonwealth of Australia 2017) has recently been created for six of the seven marine turtle species found globally, and conservation interventions are commonplace as part of an objective to support the management of marine turtle populations. The green turtle (*Chelonia mydas*) is currently IUCN red-listed as Endangered (Seminoff 2004), although this assessment is now considered out of date (i.e. more than ten years old) and therefore may be an inaccurate representation of its status. Globally, turtle rehabilitation centres seek to rescue injured or sick marine turtles, including *C. mydas*, and then rehabilitate and release recovered individuals. Turtles may remain in rehabilitation centres for periods varying from weeks to years or in worst case scenarios require permanent captive care. A study of rehabilitation facilities in Florida (USA) between 1986 and 2004 found low success rates, whereby 61.5% of turtles died in rehabilitation and only 36.8% were released back into the wild (Baker et al. 2015).

Stress associated with captivity and poor health status may contribute to low survivorship and release success (Molony et al. 2006; Vogelnest 2008; Dickens et al. 2009). Acute and chronic stress is well documented in captive

wildlife, as is the relationship between the physiological presentation of stress, and the welfare of animals housed in captive environments (Narayan et al. 2018). Stress can cause an imbalance in the physiological equilibrium of an animal, whereby resources are redirected from normal biological functions in order to cope with the negative stimuli and initiate the 'fight or flight' response (Martin et al. 2011; Dantzer et al. 2014). Short-term, acute stress is a normal aspect of survival in the wild and each animal has its own basal corticosterone level; however, a more extreme acute stress response, or long-term exposure to negative stimuli resulting in chronic stress, can be detrimental to the animal and its recovery (Narayan et al. 2012; Narayan et al. 2018). Chronic stress has the ability to compromise some biological systems, leading to fitness consequences such as immunosuppression and inappetence which can inhibit recovery progress (Dickens et al. 2010; Baxter-Gilbert et al. 2014; Refsnider et al. 2015). Continued biological response to stress can also cause impaired reproductive function and hypercortisolism, both of which can have a negative impact on post-release survival (Carlstead & Shepherson 2000; Pryce et al. 2002; Soltis et al. 2003; Narayan et al. 2012).

However, the extent to which the captive environment can cause stress in marine turtles, and how this may affect survival during rehabilitation or post-release, remains relatively unknown. It has been suggested that to improve the success of conservation interventions (such as rehabilitation for release), increased collaboration between animal welfare and conservation physiology is required (Teixeria et al. 2007). It is therefore essential that those working with marine turtles during rehabilitation are able to detect behavioural indicators of stress in order to achieve optimal welfare conditions and improve intervention success. Similarly, it is imperative that we are able to establish the severity of potential stressors associated with environmental trauma and disease (Narayan 2019). In one study of captive three-toed box turtles (*Terrapene carolina triunguis*), the attachment of a radio transmitter did not increase stress; however, levels of faecal glucocorticoid metabolite did rise significantly in both control and treatment groups during the study, which was carried out in captivity (Rittenhouse et al. 2005).

It has been suggested that the most reliable means of visualising stress includes behavioural assays in order to supplement lab-based hormonal measurements (Otovic & Hutchinson 2015). The major glucocorticoid or stress hormone in turtles (like all herpetofauna) is corticosterone (Case et al. 2005). Non-invasive methods of measuring the stress hormone corticosterone, including the use of faecal corticosterone metabolites (FCM), are becoming more prominent in the assessment of stress (Dehnhard et al. 2001; Weingrill et al. 2004; Franceschini et al. 2008; Narayan et al. 2012; Shepherdson et al. 2013; Watson et al. 2013; Narayan et al. 2018).

Animal behaviour may be used to visualise signs of stress in captive animals. Although many taxa exhibit species specific behaviours in response to acute stress, long term stress may be more likely to be manifested in stereotypic behaviour. One of the most recent examples of chronic stress and associated stereotypic behaviour in rehabilitated wildlife was reported in a study conducted on Asiatic black bears (*Ursus thibetanus*). Narayan et al. (2018) showed that newly rescued bears from bear bile farms generated significantly high levels of physiological stress and expressed stereotypic behaviour. In a study by Vaz et al. (2017) correlation between both faecal corticosterone metabolite concentration and prevalence of stereotypic behaviour was also found in captive Royal Bengal tigers (*Panthera tigris tigris*) and Indian leopards (*Panthera pardus fusca*). Stereotypic behaviour is widely linked to stress in other research (Würbel & Stauffacher 1996; Mason & Rushen 2006; Malmkvist et al. 2011), although it is also hypothesised that it can develop as a coping mechanism to reduce stress (Rushen 1993; Wechsler 1995) and so relationships between stereotyping and physiological stress biomarkers may be complex (Cooper & Nicol 1993). Whilst there continues to be some ambiguity over the definition of stereotypic behaviours, historically the repetitive and invariant manner in which a behaviour is performed, as well as a lack of any apparent function of the behaviour, is a widely accepted characteristic (Mason & Rushen 2006; McBride & Parker 2015). Repeating pathways within an enclosure (referred to as pacing) is a common stereotypic behaviour found in captive terrestrial carnivores such as bears and large felids (Poulsen et al. 1996; Lyons et al. 1997; Margulis et al. 2003; Bashaw et al. 2007; Yalcin & Aytug 2007; Miller 2012; Shepherdson et al. 2013). In an aquatic setting a comparable behaviour referred to as 'pattern swimming' has been identified in marine turtles, including green turtles (Therrien et al. 2007). It is often hypothesised that the development of this stereotypic behaviour stems from the inability to hunt, inability to escape from the enclosure, or frustration born of an insufficient range area (Poulsen et al. 1996; Lyons et al. 1997; Margulis et al. 2003; Clubb & Vickery 2006; Bashaw et al. 2007; Yalcin & Aytug 2007; Miller 2012; Shepherdson et al. 2013). Less complex and/or naturalistic captive environments are also thought to foster the development of stereotypic behaviours (Mason et al. 2013; McBride & Parker 2015), and increased enclosure size and introduction of environmental enrichment has been found to decrease prevalence in large felids (Vaz et al. 2017). Green turtles inhabit large home ranges and undertake migratory movements over large distances thus encountering wide variation and sensory stimulation within their environment (Mendonca 1983; Lutz et al. 2002), and therefore confinement in smaller and unnatural enclosures may be a contributing factor to the development of stereotypical behaviour such as pacing (Mason et al. 2013; McBride & Parker 2015). It may also represent underlying physiological stress.

The use of both behavioural and physiological markers of stress could make it possible for those working with marine turtles in a rehabilitation setting to identify stress in real-time, with possibility of early detection. Development in this area would extend to other conservation interventions such as translocation and captive breeding programmes. This study aims to identify whether repetitive pathways (as a behavioural assay used for quantifying stereotypic behaviour) are present within a small group of captive green turtles housed in a rehabilitation environment. For the first time in a marine turtle species, we also trialled the use of faecal corticosterone metabolites (FCM) as a biological marker of stress in a non-invasive alternative to the classical use of blood sampling. It is hypothesised that the rescued green turtles will show stereotypic behaviour and record detectable levels of FCMs.

Materials and methods

Study site and study animals

The study took place at Cairns Turtle Rehabilitation Centre, located on Fitzroy Island in Queensland, Australia, between June and July 2018. Four green turtles (*Chelonia mydas*) were included in the study. The subjects varied in age, sex, time in captivity and medical history (Table 1).

Turtles were housed individually in circular tanks with a surface area of 12.45m^2 (diameter = 4m), filled with sea water to a volume of 8.37m^3 (depth = 0.67m). Tanks were situated in an outdoor environment covered by a canopy, with water temperature ranging from 25-30°C during the period of data collection. Each tank was fitted with two bag filters which were removed and cleaned daily with a high-pressure hose and sea water. Turtles were fed an *ad libitum* diet of prawns, squid and whitebait daily between 09:00 and 10:00 AEST, or until appetite supressed. Feed was equally distributed around the tank by hand from a distance. Tanks were cleaned daily between 10:00 and 10:30 whilst the turtle remained in the tank, using a siphon and a fine mesh net measuring 21x16cm.

Public tours (of between 15-20 people) took place daily between 13:00 and 14:00 and involved one turtle per day. Data collection was carried out on a rolling basis, whereby the subject involved in the public tour was not observed over the 48 hours following. A hands-off policy was not in place at the facility and volunteers were encouraged to interact with the turtles, except for the two juvenile subjects. Interaction involved moving the turtle around the tank to encourage swimming, and scratching the carapace as a form of enrichment in replicating the sensation achieved through cleaning stations (Sazima et al. 2010; Monreal-Pawlowsky et al. 2017; Schofield et al. 2017).

Data collection for this study did not alter the contemporary policy, conditions and husbandry regime of the study site.

Table 1. Subject information and medical history.

Subject	Sex	Estimated age	Approximate time in captivity	Medical history	Medical interventions	Involved in public tour
M-ADULT	M	Adult	2 years	Found floating on side off the coast – severely underweight, starvation.	Force-fed at time of intake.	Y
F-ADULT	F	Adult	2 years	Stranding, starvation.	Force-fed at time of intake.	N
F-JUVENILE	F	Juvenile	1 year	Recurring stranding as a hatchling, same clutch as M-JUVENILE. Seized from a member of the public. Passed a large amount of microplastics upon intake.		Y
M-JUVENILE	M	Juvenile	1 year	Recurring stranding as a hatchling, same clutch as F-JUVENILE. Seized from a member of the public. Passed a large amount of microplastics upon intake.		Y

Repetitive pathways: zone sequence analysis

Pacing is often categorised by visual observation without parameters to identify at which point the behaviour switches from random to repetitive locomotion. Therefore, there remains a need for a method which can enable the extent of invariance to be quantified, with minimal degree of subjectivity. The probability of one behaviour following another specific behaviour is termed transition probability (Martin & Bateson 2007) and is often utilised in ethology (Verschure et al. 2003; Yang & Deb 2010; du Preez et al. 2015). If each component behaviour occurs in the same sequence this is described as deterministic. This can be quantified by using a transition matrix which provides transition probability. This model can therefore be used to define whether pathways within an enclosure are categorically repetitive and can allow the application of parameters and scale. This method, termed Zone sequence analysis, reveals 1) whether the observed transition probability differed significantly from the expected probability, 2) which pathways were actively used and 3) the increased probability that an active pathway will be taken (given the pathways available).

A GoPro HERO (GoPro Inc, USA) was affixed to the side of the tank using a suction cup mount. The camera was placed above the water surface to reduce disturbance of the animal. A pilot study was carried out over three days prior to the data collection period, during which time the camera equipment was affixed to aid habituation to the new stimulus, and to identify placement for optimal visibility.

Behavioural observations took place between 11:00 and 14:00 in one-hour slots over a period of 14 days between 18 June and 6 July 2018. Subjects were recorded on a rotation basis in order to observe each subject at different times. Each subject was observed for a total of ten hours using continuous focal sampling. The tank was divided into twelve zones (Fig. 1). Each time the subject entered a new zone (>50% of body length), the zone number was recorded.

Following each recording the footage was uploaded to a MacBook Air for analysis. Zone transitions were recorded and zone sequence analysis was applied to the extracted data. In order to determine whether pathways were repeated the transition between each zone was analysed for transition probability (TP), e.g. the probability that zone 1 will follow zone 2 rather than any other accessible zone (Martin & Bateson 2007). The sequence of zones was analysed for TP using a transition matrix (Martin & Bateson 2007) in Microsoft Excel for Mac v15.39.

These data were paired with the expected TP, calculated from a random probability model based on the zones physically accessible to the subject, i.e. only five zones can be accessed from zone 1 (2, 6, 8, 7, 12) and so the random probability for each pathway is 0.2. Only the possible pathways were

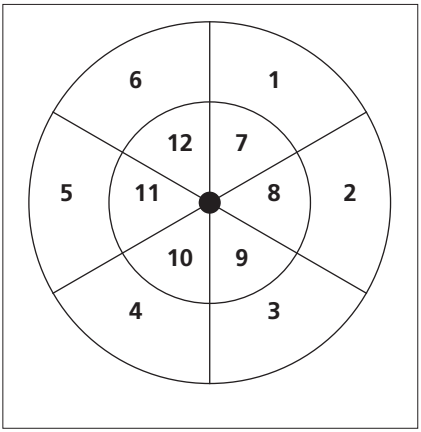
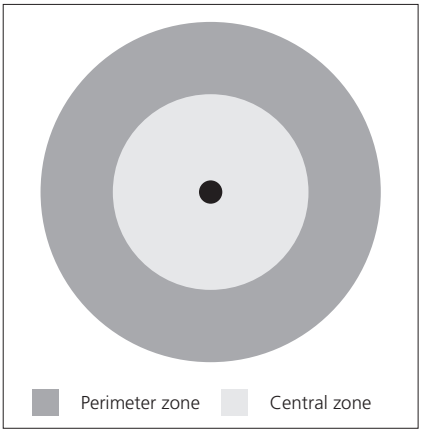


Fig. 1. a) Diagram of the tank divided into twelve zones, with an overflow pipe at the centre of the tank acting as a landmark.



b) Diagram of the tank divided into two zones, perimeter and central, with an overflow pipe at the centre of the tank acting as a landmark.

considered, i.e. a subject cannot physically pass from zone 2 to zone 5 and therefore this pathway is omitted from both datasets.

Commonly, pacing occurs around the perimeter of an enclosure (perimeter-pacing) (Poulsen et al. 1996; Lyons et al. 1997; Bashaw et al. 2007; Miller 2012; Shepherdson et al. 2013), although it has also been seen in other contexts, such as at the location of food distribution (Margulis et al. 2003; Yalcin & Aytug 2007). A secondary dataset produced from construction of the transition matrix expresses the route of each pathway using perimeter and central zones as the two variables. Therefore, the route p-p (perimeter zone to perimeter zone) can be used to illustrate the number of pathways which took place within the perimeter zone as a whole (Fig. 1), and thus followed the characteristic of 'perimeter pacing'.

Fig.1. c) Adult male green turtle (M-ADULT) in the perimeter zone of the tank at Cairns Turtle Rehabilitation Centre.

Faecal corticosterone metabolite analysis

Faecal samples were collected opportunistically over a period of 14 days using a fine mesh net and stored on site in Low Density Polyethylene 153-526 zip lock bags (Edu-Lab, UK) within a -20°C freezer on site. The maximum duration of time the sample may have remained in the tank before removal was 14 hours. At the end of the data collection period, samples were transported to the lab where they were placed within a -20°C freezer. Samples were transported on ice within an Insulated Hybrid Cooler model number 1315490 (Esky, Australia), Time outside of freezer conditions did not exceed six hours and temperature within the cooler was monitored throughout transit. Samples were assayed within one month of sampling.

Faecal corticosterone metabolites were extracted from turtle faecal samples using the methods as previously described (Narayan et al. 2013). Briefly, samples were freeze-dried or lyophilized to eliminate all water content, ground, and a small amount was extracted using aqueous methanol. This was air-dried and then reconstituted in an assay buffer before analysis by the enzyme-immunoassay (EIA).

The EIA used was originally validated in our earlier research work (Narayan et al. 2010). Briefly, concentrations of faecal corticosterone metabolites were determined using a polyclonal anticorticosterone antiserum (CJM006) as described.

For the assay, the plates were prepared with buffered antibody and faecal samples using the method described by Narayan et al. (2010). They were incubated for two hours and when the reaction was stopped the optical density (OD) was read using an ELISA plate reader (ThermoScientific Multiskan SK, Ascent software version 2.6). Faecal corticosterone metabolites levels were expressed as ng/g dry weight.

Results

Faecal corticosterone metabolite analysis

The EIA detected FCMs in the pool within 80% binding. Assay sensitivity was 2.01 ± 0.31pg well^{-1}. Recovery of corticosterone standard was 89%. Coefficient of variation for intra- and inter-run assay was 3.2% and 7.2% respectively. FCM was successfully extracted from a total of nine samples (range = 5.2-8.5ng/g, mean = 6.46 ng/g, SE = 0.38, CV% = 1.13). There was no significant difference between mean concentration across the four subjects ($X^2_3 = 3$, $p = 0.4$) (Table 2).

Zone sequence analysis

For F-JUVENILE, M-JUVENILE and F-ADULT the observed TP (TP_o) differed significantly to the expected TP (TP_e) of the random model ($X^2_{37} = 54.2$, $p = 0.03$; $X^2_{46} = 67.7$, $p = 0.02$, $X^2_{53} = 71.9$, $p = 0.04$ respectively), signifying

that these subjects repeated some pathways more frequently than others (Table 2). TP_o of M-ADULT did not differ significantly from the expected TP_e ($X^2_{52} = 62.9$, $p = 0.14$).

Table 2. Mean increase in transition probability and mean FCM concentration for each subject.

Subject	Mean increased probability (($TP_o - TP_e$))	Mean FCM concentration (ng/g)
F-JUVENILE	0.20	8.50
M-JUVENILE	0.04	5.65
M-ADULT	0.02	6.95
F-ADULT	0.03	5.25

The Markovian chain displays which pathways were repeated more frequently than others, and the increased probability that each pathway would be taken (Fig. 2). For example, from zone 3 F-JUVENILE was 44% (0.44) more likely to travel to zone 2 than any other (accessible) zone ($TP_e = 0.20$, $TP_o = 0.64$).

The mean increased probability of active pathways ($TP_o - TP_e$) indicates the extent to which each individual performed repeated pathways (Table 3), which varied from 0.02 / 2% (M-ADULT) to 0.20 / 20% (F-JUVENILE); however, there was no statistical significance in the differences between the four subjects ($X^2_3 = 3$, $p = 0.4$).

To reflect on the proportion of pathways which remained within the perimeter of the enclosure, the transition matrix (Fig. 3) can also reveal the proportion of perimeter to perimeter pathways as shown in Table 3.

Table 3. Pathways of all transitions. N is the number of pathways. R is the percentage of pathways which took this route.

	F-JUVENILE		M-JUVENILE		M-ADULT		F-ADULT	
Total transitions	1465		698		598		792	
	N	R	N	R	N	R	N	R
Central > Perimeter	38	3%	63	9%	83	14%	106	13%
Central > Central	46	3%	101	15%	211	35%	289	37%
Perimeter > Central	40	3%	64	9%	86	14%	104	13%
Perimeter > Perimeter (p-p)	1341	91%	470	67%	218	37%	293	37%

Discussion

Faecal corticosterone metabolite

One of the key aims of the study was to successfully extract FCM from a collection of temporarily captive marine turtles. Whilst there was no significant difference in mean concentration across the four subjects, this achievement is a valuable development in the endorsement of this method as an alternative to blood sampling in green turtles, and should be developed further to achieve validation and establish baseline data. To our knowledge, there is no previous published data on FCMs in marine turtles. However, in a study using a traditional blood sampling method, Hunt et al. (2016) showed that distressed leatherback turtles (*Dermochelys coriacea*) expressed over 2x higher levels of blood corticosterone compared to healthy turtles. In a study on captive three-toed box turtles in captivity, researchers did not detect a difference between control and treatment turtles before, during, or after radiotransmitter attachment, and did not find a significant relationship between time in captivity and faecal glucocorticoid metabolite levels (Rittenhouse et al. 2005). Therefore, it is can be suggested that activity of the HPI-axis and levels of glucocorticoids quantified across taxa may vary depending on the context of the stressor. Further research is needed to determine if the sea turtles were adapted to routine health checks hence why we could not find significant difference in FCM levels between individuals; however, our sample size was low to make any conclusive remarks. Similarly, by ascertaining the true gut transition time of green turtles FCM analysis may also be used to indicate stress in response to stimuli, which will be an essential next step in exploring the impact of events such as medical interventions and other human-interaction. As every animal has its own stress-hormone baseline, in order to directly relate FCM concentration to stress long-term monitoring using an inclusive and multi-disciplinary approach is essential (Touma & Palme 2005), and should be approached using physiological, biological and behavioural assays.

Zone sequence analysis

Zone sequence analysis revealed a statistically significant difference between the expected transition probability and observed transition probability for three of the four subjects. This result indicates that repetitive pathways were present in the sample population. There was no significant difference in the mean increased probability for all four subjects although this is unlikely to be conclusive given the limited sample size.

Zone sequence analysis was successful in its aim; to quantitatively identify the presence of repeated pathways. This progress in measuring stereotypic behaviour has potential to lend itself to a variety of species within any enclosure with full visibility, providing a valuable step for research involving stereotypic behaviour in aquatic species. By developing our understanding of the factors influencing behavioural and

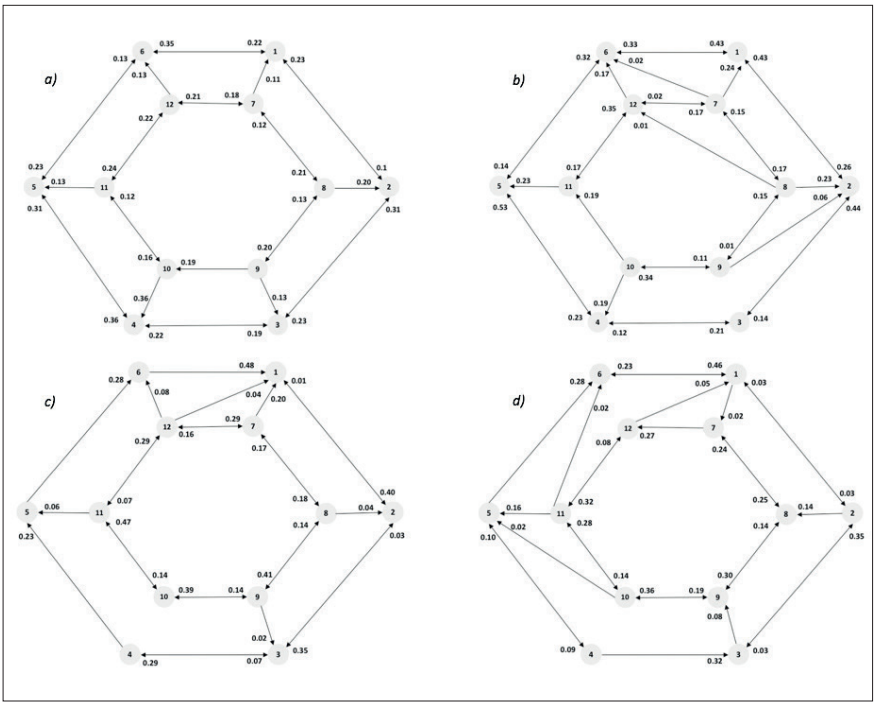


Fig. 2. Markovian chain displaying active pathways for each individual. Zones are displayed in each circle and mirror the layout as shown in figure 1. The increased probability is shown at each arrowhead. a) M-JUVENILE b) F-JUVENILE c) F-ADULT d) M-ADULT.

		START ZONE											
		Perimeter zones						Central zones					
		1	2	3	4	5	6	7	8	9	10	11	12
END ZONE	Perimeter zones 1		0.2285 (0.2)				0.658 (0.2)	0.104 (0.125)	0.06 (0.125)				0.17 (0.125)
	Perimeter zones 2	0.2325 (0.2)		0.55 (0.2)				0.02 (0.125)	0.06 (0.125)	0.0796 (0.125)			
	Perimeter zones 3		0.2285 (0.2)		0.518 (0.2)				0.04 (0.125)	0.0577 (0.125)	0.0425 (0.125)		
	Perimeter zones 4			0.103 (0.2)		0.291 (0.2)				0.0384 (0.125)	0.0851 (0.125)	0.08 (0.125)	
	Perimeter zones 5				0.296 (0.2)		0.19 (0.2)				0.1489 (0.125)	0.28 (0.125)	0.1063 (0.125)
	Perimeter zones 6	0.43 (0.2)				0.479 (0.2)		0.0833 (0.125)				0.14 (0.125)	0.1063 (0.125)
	Central zones 7	0.22 (0.2)	0.0571 (0.2)				0 (0.2)		0.36 (0.125)	0.0384 (0.125)	0 (0.125)	0.02 (0.125)	0.1276 (0.125)
	Central zones 8	0.0813 (0.2)	0.343 0.0	0 (0.2)				0.375 (0.125)		0.269 (0.125)	0 (0.125)	0 (0.125)	0.0212 (0.125)
	Central zones 9		0.14 (0.2)	0.276 (0.2)	0.074 (0.2)			0 (0.125)	0.42 (0.125)		0.319 (0.125)	0.02 (0.125)	0.0212 (0.125)
	Central zones 10			0.0689 (0.2)	0.074 (0.2)	0.0625 (0.2)		0 (0.125)	0.02 (0.125)	0.48 (0.125)		0.26 (0.125)	0 (0.125)
	Central zones 11				0.037 (0.2)	0.1458 (0.2)	0 (0.2)	0.02 (0.125)	0 (0.125)	0.0384 (0.125)	0.4 (0.125)		0.4468 (0.125)
	Central zones 12	0.035 (0.2)				0.02 (0.2)	0.15 (0.2)	0.396 (0.125)	0.04 (0.125)	0 (0.125)	0 (0.125)	0.2 (0.125)	

Fig. 3. Example of transition matrix (M-ADULT) showing TP_o probabilities, with TP_e in brackets.

physiological stress response in the green turtle, it is hoped the methods may be used to inform the best practice husbandry of captive environments. Although the current study involved a small sample size, which is often the case for non-model taxa in captive settings, expansion to a larger sample size of broader demography may elucidate the applicability of these methods.

The monitoring of pacing behaviour may be integrated within ongoing health and welfare evaluation and the decision-making process for eventual release. It will be therefore essential to consider the impact of human interaction and other potential environmental stressors, as turtle behaviour is known to be sensitive to noise pollution, light pollution and tactile interaction (Witherington & Martin 2000; Therrien et al. 2007; Weilgart 2007; Chepesuik 2009; Kamrowski et al. 2012).

Conclusion

In summary, the findings present an important opportunity to take the method of zone sequence analysis further in order to identify the factors which may influence the development of pacing in captive green turtles and other marine turtle species.

The successful extraction and analysis of corticosterone metabolites concentration via faecal sampling should be an advocate for the use of this method as a non-invasive alternative to blood sampling, and as a means to reduce stress to the animal and thus improve welfare.

It is imperative that research continues to improve our understanding of the interaction between stress physiology and the presentation of stress through behavioural indicators, and how both influence the success of conservation interventions aimed at reducing the decline of marine turtle populations.

Acknowledgements

This work was supported by the Institute of Zoology and the British Chelonia Group. The authors also wish to thank the staff and volunteers at the Cairns Turtle Rehabilitation Centre for accommodating data collection. Laboratory hormone analysis was conducted by Dr Edward Narayan at the Stress Lab. Ethics approval was obtained through the Ethical Review Board at the Zoological Society of London (Reference WAB67).

References

Baker, L., Edwards, W. & Pike, D.A. (2015). Sea turtle rehabilitation success increases with body size and differs among species. *Endangered Species Research* 29(1): 13-21.

Bashaw, M.J., Kelling, A.S., Bloomsmith, M.A. & Maple, T.L. (2007). Environmental effects on the behavior of zoo-housed lions and tigers, with a case study of the effects of a visual barrier on pacing. *Journal of Applied Animal Welfare Science* 10(2): 95-109.

Baxter-Gilbert, J.H., Riley, J.L., Mastromonaco, G.F., Litzgus, J.D. & Lesbarrères, D. (2014). A novel technique to measure chronic levels of corticosterone in turtles living around a major roadway. *Conservation Physiology* 2(1): cou036.

Carlstead, K. & Shepherdson, D. (2000). Alleviating stress in zoo animals with environmental enrichment. *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare.* Cabi, pp. 337-354.

Case, B.C., Lewbart, G.A. & Doerr, P.D. (2005). The physiological and behavioural impacts of and preference for an enriched environment in the eastern box turtle (*Terrapene carolina carolina*). *Applied Animal Behaviour Science* 92(4): 353-365.

Chepesiuk, R. (2009). Missing the dark: health effects of light pollution. *Environmental Health Perspectives* 117(1): A20.

Clubb, R. & Vickery, S. (2006). Locomotory stereotypies in carnivores: does pacing stem from hunting, ranging, or frustrated escape. *Stereotypic animal behaviour: fundamentals and applications to welfare* 2: 58-79.

Commonwealth of Australia (2017). Recovery Plan for Marine Turtles in Australia. Commonwealth of Australia. Available from: https://www.environment.gov.au/system/files/resources/46eedcfc-204b-43de-99c5-4d6f6e72704f/files/recovery-plan-marine-turtles-2017.pdf [Accessed 15 January 2018].

Cooper, J.J. & Nicol, C.J. (1993). "The 'coping' hypothesis of stereotypic behaviour": Reply. *Animal Behaviour* 45(3): 616-618.

Dantzer, B., Fletcher, Q.E., Boonstra, R. & Sheriff, M.J. (2014). Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? *Conservation Physiology* 2(1): cou023.

Dehnhard, M., Clauss, M., Lechner-Doll, M., Meyer, H.H.D. & Palme, R. (2001). Non-invasive monitoring of adrenocortical activity in roe deer (*Capreolus capreolus*) by measurement of faecal cortisol metabolites. *General and Comparative Endocrinology* 123(1): 111-120.

Dickens, M.J., Delehanty, D.J. & Romero, L.M. (2009). Stress and translocation: alterations in the stress physiology of translocated birds. *Proceedings of the Royal Society B: Biological Sciences* 276(1664): 2051-2056.

Dickens, M.J., Delehanty, D.J. & Romero, L.M. (2010). Stress: an inevitable component of animal translocation. *Biological Conservation* 143(6): 1329-1341.

du Preez, B., Hart, T., Loveridge, A.J. & Macdonald, D.W. (2015). Impact of risk on animal behaviour and habitat transition probabilities. *Animal Behaviour* 100: 22-37.

Franceschini, M.D., Rubenstein, D.I., Low, B. & Romero, L.M. (2008). Faecal glucocorticoid metabolite analysis as an indicator of stress during translocation and acclimation in an endangered large mammal, the Grevy's zebra. *Animal Conservation* 11(4): 263-269.

Gall, S.C. & Thompson, R.C. (2015). The impact of debris on marine life. *Marine Pollution Bulletin* 92(1-2): 170-179.

Hunt, K.E., Innis, C.J., Merigo, C. & Rolland, R.M. (2016). Endocrine responses to diverse stressors of capture, entanglement and stranding in leatherback turtles *(Dermochelys coriacea). Conservation Physiology* 4(1): cow022.

IUCN (2018). IUCN Red List [*online*]. Available from: http://www.iucnredlist.org [Accessed 10 January 2018].

Kamrowski, R.L., Limpus, C., Moloney, J. & Hamann, M. (2012). Coastal light pollution and marine turtles: assessing the magnitude of the problem. *Endangered Species Research* 19(1): 85-98.

Lutcavage, M.E., Plotkin, P., Witherington, B. & Lutz, P.L. (1997). Human impacts on sea turtle survival. In: Lutz, P.L. & Musick, J.A. (eds). *The Biology of Sea Turtles* (Vol. 1), CRC press, pp. 387-409.

Lutz, P.L., Musick, J.A. & Wyneken, J. (eds) (2002). *The Biology of Sea Turtles* (Vol. 2). CRC press.

Lyons, J., Young, R.J. & Deag, J.M. (1997). The effects of physical characteristics of the environment and feeding regime on the behavior of captive felids. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association* 16(1): 71-83.

Malmkvist, J., Jeppesen, L.L. & Palme, R. (2011). Stress and stereotypic behaviour in mink (*Mustela vison*): a focus on adrenocortical activity. *Stress* 14(3): 312-323.

Margulis, S.W., Hoyos, C. & Anderson, M. (2003). Effect of felid activity on zoo visitor interest. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association* 22(6): 587-599.

Martin, P. & Bateson, P. (2007). *Measuring Behaviour: An Introductory Guide*. Cambridge University Press.

Martin, L. B., Andreassi, E., Watson, W. & Coon, C. (2011). Stress and animal health: physiological mechanisms and ecological consequences. *Nature Education Knowledge* 3(6): 11.

Mason, G. & Rushen, J. (eds) (2006). *Stereotypic animal behaviour: fundamentals and applications to welfare*. Cabi.

Mason, G., Burn, C.C., Dallaire, J.A., Kroshko, J., Kinkaid, H.M. & Jeschke, J.M. (2013). Plastic animals in cages: behavioural flexibility and responses to captivity. *Animal Behaviour* 85(5): 1113-1126.

McBride, S.D. & Parker, M.O. (2015). The disrupted basal ganglia and behavioural control: an integrative cross-domain perspective of spontaneous stereotypy. *Behavioural Brain Research* 276: 45-58.

Mendonca, M.T. (1983). Movements and feeding ecology of immature green turtles (*Chelonia mydas*) in a Florida lagoon. *Copeia* 1983: 1013-1023.

Miller, L.J. (2012). Visitor reaction to pacing behavior: influence on the perception of animal care and interest in supporting zoological institutions. *Zoo Biology* 31(2): 242-248.

Molony, S.E., Dowding, C.V., Baker, P.J., Cuthill, I.C. & Harris, S. (2006). The effect of translocation and temporary captivity on wildlife rehabilitation success: an experimental study using European hedgehogs (*Erinaceus europaeus*). *Biological Conservation* 130(4): 530-537.

Monreal-Pawlowsky, T., Marco-Cabedo, V., Palencia Membrive, G., Sanjosé, J., Fuentes, O., Jiménez, E. & Manteca, X. (2017). Environmental enrichment facilitates release and survival of an injured loggerhead sea turtle (*Caretta caretta*) after ten years in captivity. *Journal of Zoo and Aquarium Research* 5(4): 39.

Narayan, E. J., Molinia, F. C., Christi, K. S., Morley, C. G. & Cockrem, J. F. (2010). Annual cycles of urinary reproductive steroid concentrations in wild and captive endangered Fijian ground frogs (*Platymantis vitiana*). *General and Comparative Endocrinology* 166: 172-179.

Narayan, E., Hero, J.M., Evans, N., Nicolson, V. & Mucci, A. (2012). Non-invasive evaluation of physiological stress hormone responses in a captive population of the greater bilby *Macrotis lagotis*. *Endangered Species Research* 18(3): 279-289.

Narayan, E., Webster, K., Nicolson, V. & Hero, J-M. (2013). Noninvasive evaluation of physiological stress in an iconic Australian marsupial: the Koala (*Phascolarctos cinereus*). *General and Comparative Endocrinology* 187: 39-47.

Narayan, E., Willis, A., Thompson, R., Hunter-Ishikawa, M. & Bendixsen, T. (2018). Evaluating physiological stress in Asiatic black bears (*Ursus thibetanus*) rescued from bile farms in Vietnam. *Animal Welfare* 27(4): 295-303.

Narayan, E. (2019). Physiological stress levels in wild koala sub-populations facing anthropogenic induced environmental trauma and disease. *Scientific Reports* 9(1): 6031.

Otovic, P. & Hutchinson, E. (2015). Limits to using HPA axis activity as an indication of animal welfare. *ALTEX-Alternatives to animal experimentation* 32(1): 41-50.

Poulsen, E.M., Honeyman, V., Valentine, P.A. & Teskey, G.C. (1996). Use of fluoxetine for the treatment of stereotypical pacing behavior in a captive polar bear. *Journal of the American Veterinary Medical Association* 209: 1470-1474.

Pryce, C.R., Palme, R. & Feldon, J. (2002). Development of pituitary-adrenal endocrine function in the marmoset monkey: infant hypercortisolism is the norm. *Journal of Clinical Endocrinology & Metabolism* 87(2): 691-699.

Rees, A.F., Alfaro-Shigueto, J., Barata, P.C.R., Bjorndal, K.A., Bolten, A.B., Bourjea, J., Broderick, A.C. et al. (2016). Are we working towards global research priorities for management and conservation of sea turtles? *Endangered Species Research* 31: 337-382.

Refsnider, J.M., Palacios, M.G., Reding, D.M. & Bronikowski, A.M. (2015). Effects of a novel climate on stress response and immune function in painted turtles (*Chrysemys picta*). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* 323(3): 160-168.

Rittenhouse, C.D., Millspaugh, J.J., Washburn, B.E. & Hubbard, M.W. (2005). Effects of radio transmitters on faecal glucocorticoid metabolite levels of three-toed box turtles in captivity. *Wildlife Society Bulletin* 33(2): 706-713.

Rushen, J. (1993). The "coping" hypothesis of stereotypic behaviour. *Animal Behaviour* 45(3): 613-615.

Santos, R.G., Andrades, R., Boldrini, M.A. & Martins, A.S. (2015). Debris ingestion by juvenile marine turtles: an underestimated problem. *Marine Pollution Bulletin* 93(1-2): 37-43.

Sazima, C., Grossman, A. & Sazima, I. (2010). Turtle cleaners: reef fishes foraging on epibionts of sea turtles in the tropical Southwestern Atlantic, with a summary of this association type. *Neotropical Ichthyology* 8(1): 187-192.

Schofield, G., Papafitsoros, K., Haughey, R. & Katselidis, K. (2017). Aerial and underwater surveys reveal temporal variation in cleaning-station use by sea turtles at a temperate breeding area. *Marine Ecology Progress Series* 575: 153-164.

Seminoff, J.A. (Southwest Fisheries Science Center, U.S.) (2004). *Chelonia mydas*. *The IUCN Red List of Threatened Species* 2004: e.T4615A11037468. http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T4615A11037468.en. Downloaded on 06 November 2018.

Shepherdson, D., Lewis, K.D., Carlstead, K., Bauman, J. & Perrin, N. (2013). Individual and environmental factors associated with stereotypic behavior and faecal glucocorticoid metabolite levels in zoo housed polar bears. *Applied Animal Behaviour Science* 147(3-4): 268-277.

Soltis, J., Wegner, F.H. & Newman, J.D. (2003). Adult cortisol response to immature offspring play in captive squirrel monkeys. *Physiology & Behaviour* 80(2-3): 217-223.

Teixeira, C.P., De Azevedo, C.S., Mendl, M., Cipreste, C.F. & Young, R.J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. *Animal Behaviour* 73(1): 1-13.

Therrien, C.L., Gaster, L., Cunningham-Smith, P. & Manire, C.A. (2007). Experimental evaluation of environmental enrichment of sea turtles. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association* 26(5):407-416.

Touma, C. & Palme, R. (2005). Measuring faecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Annals of the New York Academy of Sciences* 1046(1): 54-74.

Varghese, R.M. (2015). Survey and Assessment of the Status, Issues & Threats to Marine Turtles Along the Kerala Coast. *Biodiversity Conservation – Challenges for the Future*, p. 195.

Vaz, J., Narayan, E.J., Kumar, R.D., Thenmozhi, K., Thiyagesan, K. & Baskaran, N. (2017). Prevalence and determinants of stereotypic behaviours and physiological stress among tigers and leopards in Indian zoos. *PLoS ONE* 12(4): e0174711.

Verschure, P.F., Voegtlin, T. & Douglas, R.J. (2003). Environmentally mediated synergy between perception and behaviour in mobile robots. *Nature* 425(6958): 620.

Vogelnest, L. (2008). Veterinary considerations for the rescue, treatment, rehabilitation and release of wildlife. *Medicine of Australian Mammals,* pp. 1-12.

Watson, R., Munro, C., Edwards, K.L., Norton, V., Brown, J.L. & Walker, S.L. (2013). Development of a versatile enzyme immunoassay for non-invasive assessment of glucocorticoid metabolites in a diversity of taxonomic species. *General and Comparative Endocrinology* 186: 16-24.

Wechsler, B. (1995). Coping and coping strategies: a behavioural view. *Applied Animal Behaviour Science* 43(2): 123-134.

Weilgart, L.S. (2007). The impact of ocean noise pollution on marine biodiversity. *International Ocean Noise Coalition*, pp. 501-508.

Weingrill, T., Gray, D.A., Barrett, L. & Henzi, S.P. (2004). Faecal cortisol levels in free-ranging female chacma baboons: relationship to dominance, reproductive state and environmental factors. *Hormones and Behavior* 45(4): 259-269.

Witherington, B.E. & Martin, R.E. (2000). *Understanding, Assessing, and Resolving Light-Pollution Problems on Sea Turtle Nesting Beaches*. St. Petersburg, FL: Florida Marine Research Institute.

Würbel, H. & Stauffacher, M. (1996). Prevention of stereotypy in laboratory mice: effects on stress physiology and behaviour. *Physiology & Behavior* 59(6): 1163-1170.

Yalcin, E. & Aytug, N. (2007). Use of fluoxetine to treat stereotypical pacing behavior in a brown bear (*Ursus arctos*). *Journal of Veterinary Behavior: Clinical Applications and Research* 2(3): 73-76.

Yang, X.S. & Deb, S. (2010). Engineering optimisation by cuckoo search. *International Journal of Mathematical Modelling and Numerical Optimisation* 1(4): 330-343.

