THE ROYAL SOCIETY biology letters

Reproduction in shark-attacked sea turtles is supported by stress-reduction mechanisms

Tim Jessop[1*], Joanna Sumner[1], Val Lance[1] and Col Limpus[2]

[1]*Center for Reproduction of Endangered Species, Zoological Society of San Diego, San Diego, CA 92112, USA*
[2]*Queensland Department of Environment, PO Box 155, Brisbane, QLD 4002, Australia*
[*] *Author for correspondence* (*tjessop@sandiegozoo.org*).

Recd 11.08.03; *Accptd* 10.09.03; *Online* 30.10.03

Vertebrates exhibit varied behavioural and physiological tactics to promote reproductive success. We examined mechanisms that could enable female loggerhead turtles to undertake nesting activities and maintain seasonal reproduction despite recent shark injuries of varying severity. We proposed that endocrinal mechanisms that regulate both a turtle's stress response and reproductive ability are modified to promote successful and continued reproduction. Irrespective of the degree of injury, females did not exhibit increased levels of the stress hormone corticosterone, nor decreased levels of the reproductive steroid testosterone; hormone responses consistent with stress. When exposed to a capture stressor, females with shark injury did not exhibit any greater corticosterone response than controls. In addition, breeding females showed a reduced corticosterone stress response compared to non-breeding females. Reduced endocrinal responses following shark injury, and during breeding in general may, in part, enable females to maintain behavioural and physiological commitment to reproduction.

Keywords: reproduction; reproductive commitment; shark injury; hormones; *Caretta caretta*

1. INTRODUCTION

During reproduction, organisms may use specific behavioural and physiological tactics to maximize fitness (Stearns 1976; Roff 1992). Such tactics generally increase the reproductive commitment of animals and can contribute to reproductive success. One aspect of reproductive commitment is demonstrated when organisms are exposed to challenges or stressors including competition, predation attempts, pervasive weather phenomena, energetic limitations or disease. In such instances it appears that breeding animals can actively promote or endure stressors and thus take risks to maximize their reproductive success, even if such actions could lead to injury and mortality and impart fitness costs (Stearns 1976; Roff 1992). The capacity for vertebrates to promote physiological and behavioural commitment during reproduction involves regulation from proximal mechanisms. Steroid hormones have been implicated in mediating multiple physiological and behavioural traits underpinning reproductive tactics and strategies that promote reproductive success (Marler & Moore 1988; Sinervo & Svensson 1998; Ketterson & Nolan 1999). We provide evidence for how the steroid hormones corticosterone and testosterone may facilitate reproductive commitment and thus maintain reproduction in loggerhead turtles (*Caretta caretta*) that have been subject to shark attack.

During the two to three month breeding phase, female loggerhead turtles nesting on the Swain's islands in the Great Barrier Reef are exposed to attack from tiger sharks (*Galeocerdo cuvier*), a major predator of this species (Heithaus *et al.* 2002). These attacks result in a continuum of injury ranging from superficial lacerations, to severe injury and fatal attacks. Despite injuries, even those that must constitute a major physiological and chronic stress to the animal, female loggerhead turtles do not easily abandon their immediate nesting episode or subsequent seasonal reproductive activities. For example, we documented a breeding female loggerhead nesting with a recent severe shark-inflicted injury; a large portion of the female's abdominal cavity had been bitten off, exposing viscera that dragged along the sand as she ascended the beach, where she successfully oviposited.

Few studies have investigated the potential mechanisms facilitating and maintaining reproduction in injured vertebrates. Specific glucocorticoid stress and sex-steroid hormone responses could be partly responsible for maintaining reproduction in the face of such injuries (Wingfield *et al.* 1998). We predicted that for loggerhead turtles to persist with reproduction despite shark injury they might possess the capacity to downregulate or desensitize their adrenocortical stress response. Such a mechanism could promote reproductive risk taking by preventing the steroid hormone corticosterone from inducing specific defensive physiological and behavioural mechanisms that might interfere with reproduction (Wingfield *et al.* 1998; Sapolsky *et al.* 2000). Further, the reproductive axis of these females must remain intact for subsequent inter-season nesting events to continue post-injury. We predicted that these females would exhibit no significant elevation in basal levels of the stress hormone corticosterone, nor a reduction in the sex steroid testosterone, a physiological marker of reproductive activity, irrespective of the severity of shark-related injury. Elevation of basal levels of corticosterone for sustained periods, as a result of stressors, is known to have both pathological and negative reproductive consequences for a wide variety of vertebrates (Sapolsky *et al.* 2000). Second, given the capacity of these shark-injured females to persist with reproduction, we predicted that such females would not alter their endocrine response for corticosterone and testosterone following a subsequent capture stressor, similar to uninjured control females. Typically, injured or diseased animals exhibit greater responsiveness in their stress response, as a mechanism to maintain homeostasis that is already subjected to increased allostatic loading (McEwen & Wingfield 2003).

Finally, we compared maximal concentrations of plasma corticosterone for shark-injured nesting turtles, non-injured nesting turtles and a group of non-breeding adult females all exposed to a capture stressor. This comparison allowed us to detail the suspected reduction in adrenocortical responsiveness of nesting turtles (control and shark-attacked) compared to non-breeding female

DOI 10.1098/rsbl.2003.0102

Table 1. Plasma levels of corticosterone and testosterone in the loggerhead turtle for each of six levels of shark injury rated according to severity from least to most severe.
(*n* indicates sample size.)

injury category	corticosterone (ng ml^{-1})	testosterone (ng ml^{-1})	*n*
control	3.82 ± 1.24	1.66 ± 0.30	9
superficial	2.78 ± 0.57	2.38 ± 0.59	6
minor	3.39 ± 0.79	1.45 ± 0.76	3
moderate	3.17 ± 1.07	2.22 ± 0.38	3
major	—	—	0
extensive	3.24	1.98	1

loggerhead turtles, and thus could provide additional documentation of the dramatic difference in stress responsiveness associated with reproductive status, a trait observed in other marine turtles (Jessop *et al.* 2000; Jessop 2001).

Briefly, we proposed that deliberately unresponsive endocrine mechanisms may, in part, underpin the behavioural and physiological tenacity of female loggerhead turtles in persisting with reproduction despite shark-inflicted injury.

2. MATERIAL AND METHODS

Fieldwork was conducted around nesting islands in the Swain Reefs in the Great Barrier Reef. Female loggerhead turtles were captured in water using a boat or by jumping on inter-nesting females as they swam close to the beach. Immediately after capture (less than 2 min) a blood sample was taken and females were assessed for recent shark injury. Only injuries that were less than approximately two weeks old were included, indicated by the absence of wound closure or significant fibrin deposition associated with haemorrhage. Shark injury was indexed on a six-level severity rating: (i) *control*—no shark-inflicted injury (or other injury); (ii) *superficial*—injury to plastron, carapace or head causing superficial rakings to hard, keratinized and bony areas, no injury to soft tissue; (iii) *minor*—injury to front and/or back flippers, resulting in lacerations up to 5–10 cm long and 1–2 cm deep with minor haemorrhage; (iv) *moderate*—injury causing lacerations to soft tissue areas around shoulder/pectoral and inguinal regions or partial loss of front or hind flippers (less than 50%), lacerations long and deep, greater than 5 cm in length and 2 cm in depth; (v) *major*—recent attack causing substantial loss (greater than 50%) of front or hind flippers; (vi) *extensive*—severe recent attack resulting in partial loss of carapace or plastron, exposing or damaging internal viscera.

(a) *Capture stress protocol*

Groups of uninjured control ($n = 5$) and shark-injured loggerhead turtles ($n = 5$) were exposed to a capture stress protocol involving handling and restraint (*sensu* Wingfield) to ascertain if shark-injured turtles exhibited differences in profiles of corticosterone and testosterone when compared to controls. The capture stress protocol involved capturing inter-nesting female turtles, hauling them onto the beach and placing them on their backs for 5 h. This form of capture stress has been used in marine turtles to induce an adrenocortical stress response (Jessop 2001). Each female was bled at 0, 3 and 5 h after capture then released back to the ocean. Handling and restraint, as described above, provides a standardized stressful stimulus that is consistent for all turtles. To compare the magnitude of the stress response during reproduction with non-reproductive phases of the adult female life history, a group of non-breeding adult females ($n = 7$) captured outside the breeding season was included.

Plasma samples from *C. caretta* were analysed by specific radioimmunoassays for corticosterone and testosterone. All samples for each hormone were measured in a single assay to avoid inter-assay variation. The average intra-assay coefficient of variation was 10.3% and 12.3% for corticosterone and testosterone, respectively. The resulting hormone data for corticosterone and testosterone did not significantly deviate from normality. We therefore tested the effects of injury and holding time during the capture stress protocol on these variables using parametric methods. A one-way ANOVA was performed to determine whether differences in reproductive status affected the magnitude of corticosterone responses to capture stress (corticosterone levels at 5 h post-capture versus time 0) between breeding females (both injured and uninjured) and non-breeding females.

3. RESULTS

Nesting loggerhead turtles subject to shark injury did not exhibit elevated plasma levels of the stress hormone corticosterone irrespective of the severity of the injury (ANOVA: $F_{3,23} = 0.19$, $p = 0.90$). Even the single nesting female in the highest category of shark injury (excluded from ANOVA analysis) possessed plasma levels of corticosterone within the range exhibited by less injured females (table 1). Similarly, there was no significant change in plasma levels of testosterone with severity of injury in shark-injured loggerheads (ANOVA: $F_{3,23} = 1.08$, $p = 0.38$).

When exposed to the capture stress protocol, both shark-injured and control females significantly increased plasma corticosterone levels over the 5 h of the capture stress protocol (ANOVA: $F_{2,29} = 8.54$, $p < 0.01$). However, despite their injuries, shark-attacked females exhibited no greater capacity to respond to the capture stress protocol than uninjured control females (ANOVA: $F_{2,29} = 0.01$, $p = 0.63$; figure 1). The magnitude of the corticosterone stress response after 5 h of capture stress in both groups of breeding females was significantly lower than non-breeding females captured outside the breeding season (ANOVA: $F_{2,16} = 12.04$, $p \leqslant 0.001$; figure 2), suggesting that the active reproductive state of nesting females may result in a reduced capacity to respond physiologically to stressors.

4. DISCUSSION

Nesting loggerhead turtles, despite extreme and recent shark injury, can maintain reproduction during the immediate nesting event and, given the prevalence of nesting females carrying injuries greater than two weeks old (the average inter-nesting period), can maintain reproduction throughout the breeding season. Despite their injuries, females did not have elevated levels of corticosterone, nor decreased levels of testosterone, physiological indicators of endocrinal mechanisms that could promote reproduction despite such injuries. Further, the active reproductive state of females is associated with a decreased capacity for corticosterone stress responsiveness compared to non-breeding females. Limited corticosterone levels suggest that female turtles are decreasing their acute stress response to a less responsive state. Thus, we suspect that

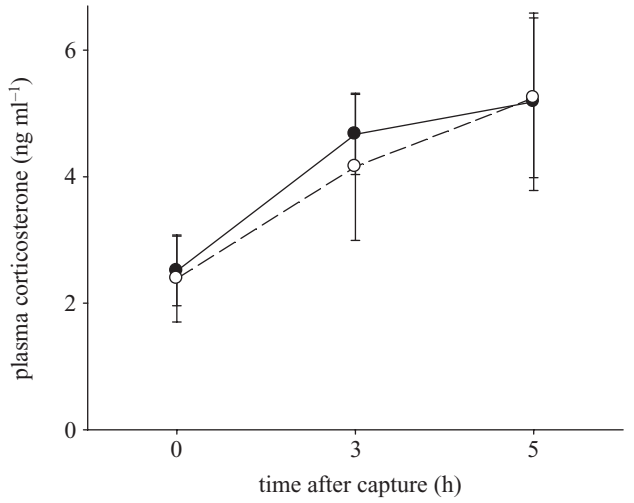

Figure 1. Plasma corticosterone levels for nesting loggerhead turtles exposed to a capture stress protocol. Shark-injured females (filled circles, $n = 5$) do not differ in their corticosterone levels compared to non-injured control females (open circles, $n = 5$). Error bars show s.e.

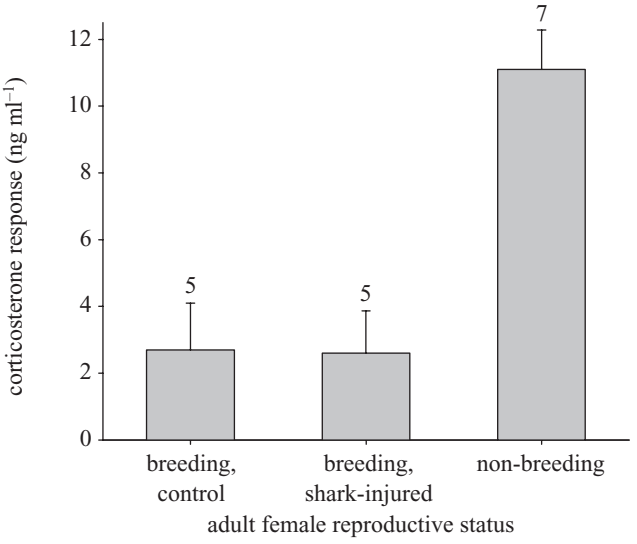

Figure 2. Plasma corticosterone levels indicate that breeding female loggerhead turtle stress responses (control and shark-injured) are significantly reduced compared to non-breeding females. Error bars show s.e. with sample size (n) above.

through the actions of steroid hormones, corticosterone in particular, females might mediate several coordinated physiological and behavioural mechanisms promoting reproduction via increased risk taking. Typically, when vertebrates are exposed to stressors in their environment they increase glucocorticoid levels to modify ongoing behavioural and physiological actions into immediate life-saving activities, often at the expense of reproduction (Wingfield *et al.* 1998; Sapolsky *et al.* 2000). However, a decrease in the stress responsiveness of the hypothalamic–pituitary–adrenal axis to external stimuli by reducing the capacity of breeding females to produce corticosterone could eliminate or reduce such defensive actions that could interfere with reproduction (Wingfield *et al.* 1998).

A reduction in corticosterone response to stressors in female loggerheads carrying shark injuries could facilitate reproduction by several mechanisms. Increased corticosterone during stress results in the mobilization of stored energy reserves (carbohydrate and protein). This energy, which if used for maintenance, could necessitate catabolism of somatic resources used during the reproductive period, or possibly induce atresia of mature follicles, and thus competes for resources necessary to sustain reproduction (Sapolsky *et al.* 2000; Wingfield & Kitaysky 2002). Further, if acute and particularly chronic high levels of corticosterone were maintained in shark-injured females it might have temporary, or possibly permanent, inhibitory effects on reproduction through both behavioural and physiological pathways (Sapolsky *et al.* 2000). Thus, a reduction in basal and stress-activated stress hormones during such perturbations enables organisms to elicit an anti-stress repertoire to facilitate the current life-history state independent of environmental disturbances (Wingfield & Kitaysky 2002).

Other vertebrates are known to downregulate their response to stressors by inhibiting the release of corticosterone or decreasing their sensitivity to the actions of this steroid (Wingfield *et al.* 1998). Several birds that breed in the Arctic decrease their corticosterone stress response to promote reproduction in an environment where the temporal window for successful offspring rearing is short and may be interrupted by pervasive storms (Wingfield *et al.* 1998). This stress desensitization appears to be threshold dependent, so that if the pervasiveness and duration of the stressor increases, the adrenocortical stress response reactivates triggering behaviour and physiological events that promote survival, but which can compromise reproduction. Such a recovery mechanism appears to prevent this anti-stress response from being maladaptive and inducing survival costs (Wingfield *et al.* 1998). While a threshold recovery to adrenocortical responsiveness to stress may be adaptive for some species and particular life-history scenarios, we speculate that it may be adaptive for loggerhead turtles to maintain a robust reduction in their stress response irrespective of the severity of the shark injury. Female sea turtles exhibit a life-history pattern with a delayed age to maturity and limited lifetime breeding opportunities, these characters may have necessitated the actions of steroid hormones to elicit extreme behavioural and physiological tactics to ensure reproductive success (Jessop 2001).

In summary, nesting loggerhead turtles exhibited a reduced endocrinal response to shark injury and a capture stressor. These deliberate physiological actions could promote reproductive success by preventing environmental or ecological disturbances from interfering with reproduction. Yet, depending on the severity or magnitude of the stressor, such anti-stress mechanisms may increase costs of reproduction in female loggerhead turtles (Jessop 2001).

Acknowledgements

The authors thank the Great Barrier Reef Marine Park Authority and Queensland Parks and Wildlife Service for providing logistical support during our fieldwork.

Heithaus, M. R., Frid, A. & Dill, L. M. 2002 Shark-inflicted injury frequencies, escape ability and habitat use of green and loggerhead turtles. *Mar. Biol.* **140**, 229–236.

Jessop, T. S. 2001 Modulation of the adrenocortical stress response in marine turtles (Cheloniidae): evidence for a hormonal tactic maximizing maternal reproductive investment. *J. Zool. Lond.* **254**, 57–65.

Jessop, T. S., Hamann, M., Read, M. A. & Limpus, C. J. 2000 Evidence for a hormonal tactic maximising green turtle reproduction in response to a pervasive ecological stressor. *Gen. Comp. Endocrinol.* **118**, 107–117.

Ketterson, E. D. & Nolan Jr, V. 1999 Adaptation, exaptation, and constraint: a hormonal perspective. *Am. Nat.* **154**, S4–S25.

McEwen, B. S. & Wingfield, J. C. 2003 The concept of allostasis in biology and biomedicine. *Horm. Behav.* **43**, 2–15.

Marler, C. A. & Moore, M. C. 1988 Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. *Behav. Ecol. Sociobiol.* **23**, 21–26.

Roff, D. 1992 *The evolution of life histories*. London: Chapman & Hall.

Sapolsky, R. M., Romero, L. M. & Munck, A. U. 2000 How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preperative actions. *Endocrinol Rev.* **21**, 55–89.

Sinervo, B. & Svensson, E. 1998 Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos* **83**, 432–442.

Stearns, S. C. 1976 Life-history tactics: a review of the ideas. *Q. Rev. Biol.* **51**, 272–278.

Wingfield, J. C. & Kitaysky, A. S. 2002 Endocrine responses to unpredictable environmental events: stress or anti-stress hormones. *Integr. Comp. Biol.* **42**, 600–609.

Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M. & Richardson, R. D. 1998 Ecological bases of hormone-behavior interactions: the 'emergency life history stage'. *Am. Zool.* **38**, 191–206.