



## Research paper

## Endocrine indicators of a stress response in nesting diamondback terrapins to shoreline barriers in Barnegat Bay, NJ

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## ABSTRACT

Anthropogenic stressors such as habitat loss are a global problem for wildlife. Coastal development in the United States has replaced estuary shorelines with hard erosion barriers. In Barnegat Bay, New Jersey, the diamondback terrapin (*Malaclemys terrapin*) encounters these barriers when approaching upland beaches for nesting. To determine the effects of shoreline barriers on this threatened species' nesting abilities, we measured adrenocortical response (i.e., stress response) by comparing natural corticosterone and testosterone levels of 91 terrapins following *in situ* exposure to either an experimentally blocked, or open nesting beach. In addition, we exposed 15 individuals, from various nesting beaches, to handling stress to identify acute corticosterone secretion, finding a significant increase over 60 min to 8 ng/ml. Corticosterone did not reach this level in terrapins exposed to barriers. Corticosterone and testosterone levels were not significantly higher among terrapins exposed to barriers compared to those at open reference beaches. This lack of a stress response suggests that terrapins do not physiologically respond to barriers when they approach nesting beaches and thus are not stressed. This may be due to an adaptive trait to help female turtles complete the nesting process despite the natural stresses inherent to coming on land. Our study suggests that this lack of stress response is also applied to non-natural, human made nesting barriers. If terrapins are not physiologically capable of adapting to shoreline barriers, future erosion control structures could support terrapin nesting with periodic upland access points. This endocrinological study provides a more quantitative approach to guiding management of anthropogenic stressors upon wildlife.

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## 1. Introduction

It is well known that external perturbations trigger a stress response in vertebrates, taking the form of a glucocorticoid release (Gregory et al., 1996; Valverde et al., 1999; Lance and Rostal, 2002; Davis et al., 2008). Steroid hormones promote this adaptive stress response that enhances survival in organisms coping with natural (Wingfield et al., 1998; Jessop, 1999) and anthropogenic (French et al., 2010; Crino et al., 2011) environmental disturbances. In reptiles, the physiological consequences of the neurohormonal stress response to a chronic stressor include compromised immune responses, repressed growth, and disruption of reproductive hormone function (Mahmoud and Licht, 1997; Lance et al., 2001;

Berger et al., 2005; French et al., 2010). In order to quantify this hormonal cascade and its various effects, endogenous rhythms of the hypothalamo-pituitary-adrenal (HPA) axis must be measured for each specific species of concern (Rostal et al., 2001), as daily cycles of glucocorticoid secretion will confound the basal activity of the HPA in response to a specific stressor (Valverde et al., 1999). Although well documented with sea turtles (Rostal et al., 2001) and tortoises (Lance et al., 2001) the reproductive endocrinology of diamondback terrapins (*Malaclemys terrapin*) remains relatively unknown.

The diamondback terrapin is an estuarine turtle found along the Atlantic and Gulf coasts of the United States (Cash et al., 1997). Terrapin populations are in decline throughout their range due to multiple anthropogenic activities (Gibbons et al., 2001; Cecala et al., 2009; Harden et al., 2009). Of particular concern, human development upon terrapin nesting beaches threatens long-term population viability through its direct negative effect upon the reproductive success of this threatened species (Sheridan et al.,

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2010; Roosenburg et al., 2014) Terrapins emerge onto high-dune habitat for nesting annually, exhibiting high levels of fidelity to their natal beaches (Burger and Montevecchi, 1975; Butler et al., 2004; Szerlag-Egger and McRobert, 2007; Sheridan et al., 2010). As shoreline development and subsequent vertical erosion prevention barrier construction (such as bulkheading, rip-rap, revetments, seawalls, groins, and jetties) continues at accelerated rates, the fidelic sites that terrapins require for reproduction are becoming less accessible. For example, over the past thirty years, Barnegat Bay Estuary, New Jersey, has displayed the highest coastal development rate of any mid-Atlantic estuary, with bulkheading replacing 36% of natural shorelines (Jivoff, 2007). While the negative ecological impacts upon intertidal saltmarsh species are well studied (Able et al., 1998; Weis et al., 1998; Seitz et al., 2006; Long et al., 2011), few studies have investigated the physiological impacts of these hard shoreline barriers on wildlife moving between aquatic and terrestrial habitats, and none have specifically measured species' stress responses.

The purpose of this study was to determine whether shoreline barriers elicited significant stress responses in reproductive diamondback terrapins at their fidelic nesting beaches. We measured baseline corticosterone (CORT) and testosterone (T) levels to quantify stress of reproductive female terrapins in relation to modelled barriers. CORT levels provided a primary indication of a stress response, and T levels provided signs of reproductive hormone fluctuation due to potential stress. We hypothesized that terrapins interacting with shoreline barriers would display a higher stress response than those encountering an unobstructed nesting habitat.

## 2. Material and methods

### 2.1. Site selection and experimental manipulation

Our study sites included two terrapin nesting areas in Barnegat Bay, New Jersey, USA (39°47'N, 74°9'W): Conklin Island, within the Edwin B. Forsythe National Wildlife Refuge, and Sedge Island, one kilometer west of Island Beach State Park (Fig. 1A). The linear distance between Conklin and Sedge Islands is approximately 8 km. We selected these sites based on their historically high levels of nesting activity. Wnek (2010) reported a mean of 90 individual female terrapins arriving to nest annually at Sedge Island, with an estimated population size of  $430 \pm 23$  total individuals.

Unpublished nest depredation surveys at Conklin Island indicated over 100 nests per kilometer between 2006 and 2011 (H. Avery, personal communication). Although similar in their valuable nesting habitat, Conklin and Sedge Islands vary in their size and shape (Fig. 1A). Conklin Island is more remote, whereas Sedge Island houses an active educational facility. Because of the differences in size, shape, and levels of human activity, we refrained from making statistical comparisons of terrapin hormone levels between Sedge and Conklin Islands.

At both Sedge and Conklin Islands, we determined two similar beaches with high nesting activity (Wnek, 2010; Avery unpublished data), and identified the locations of greatest nesting terrapin emergence frequency. Experimental manipulation of these beaches included blocking the area of maximum nesting activity with Yodock™ plastic construction barriers (The Yodock Wall Company INC., Model 2001M (Metropolitan),  $182 \times 45 \times 81$  cm), as described in Winters et al. (2015). Barriers blocked 25% of the total experimental beach length at each site (Conklin 67.7 m, Sedge 20.1 m). The second designated beach was not modified or obstructed, and served as a control beach (Fig. 1B; Winters et al., 2015).

### 2.2. Blood collection

From June through July 2011, we conducted daily simultaneous surveys at experimental and control beaches on Sedge and Conklin Islands for six hours each day (approx. 9:00–15:00). All of the analyses reported in this study were conducted using blood samples from terrapins nesting at these survey beaches. Investigators sat in an inconspicuous location among *Phragmites* sp. while monitoring emergence of nesting terrapins. Upon emergence, investigators collected female terrapins after either one minute of attempted oviposition, or after completed oviposition. Heparinized blood samples (2 ml) were collected within 5 min of capture through subcarapacial venipuncture (Sheridan et al., 2010) using a 23-gauge needle. We randomly selected 15 of the captured nesting terrapins – despite site or barrier treatment – for successive bleeding at 30 and 60 min post-capture to measure acute (sudden onset) CORT response to handling. Terrapins rested in a 19-liter plastic bucket between sampling intervals. We released all terrapins at their capture location. We stored blood samples on ice for 3–6 h prior to centrifuging for 5 min at 3300 RPM (Fisher Scientific



**Fig. 1.** Study Location (A) in Barnegat Bay, NJ with terrapin nesting survey sites, and Experimental Design (B) demonstrating beaches of closure (experimental) and remaining as is (control) at both Sedge and Conklin Islands.

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