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RESEARCH

Severe Effects of Low-Level Oil Contamination on Wildlife Predicted by the Corticosterone-Stress Response: Preliminary Data and a Research Agenda

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Low-level contamination events are common but often neglected because they may not immediately harm the environment or wildlife. We suggest that even seemingly benign oil spills may cause severe damage for wildlife and we offer a rapid assessment tool for the health status of a wildlife population that is potentially affected by a spill. We studied two island populations of seagoing lizards, marine iguanas (*Amblyrhynchus cristatus*), for 20 years before a low-level oil spill hit the shores of the Galápagos archipelago in January 2001. Under natural conditions, the main cause of mortality for marine iguanas is starvation caused by a decline in their staple food, marine algae. Starvation induces a strong stress response during which the hormone corticosterone is secreted. When iguanas of one island were exposed to low-level oil contamination of their food after the tanker "Jessica" grounded, they also showed a strong stress response, allowing us to predict high mortality rates for oiled individuals. Indeed, one year after the spill 62% of the iguanas on the oiled island had died, while none of the control iguanas on the non-oiled island died. We hypothesize that oiled iguanas died from starvation because their digestive gut bacteria were killed by oil residues in their algae food. Although the exact causation of oil-induced mortality is unknown and should be investigated further, we suggest that the corticosterone-stress response is a powerful predictor for the rapid assessment of wildlife health. Low-level oil contamination appears to be a serious threat to wildlife.

Keywords: **Oil contamination, mortality, starvation, food web effects, corticosterone-stress response, marine iguanas, *Amblyrhynchus cristatus*, Galápagos islands, Jessica oil spill**

Corticosterone-Stress Response

The vertebrate stress response helps protect an organism from an external noxious stimulus, called a stressor because it elicits a stress response. One of the hallmarks of the stress response is the release of a class of steroid hormones called glucocorticoids several minutes after exposure to a stressor. All vertebrates, including fish, amphibians, reptiles, birds, and

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mammals, will elevate glucocorticoids in the blood when exposed to stress, so stressors for one species are likely to be stressors to many species, including humans.

Glucocorticoids are crucial for survival since even mild stressors can cause death in animals that lack these hormones (Darlington *et al.*, 1990). Glucocorticoids provide enormous short-term benefits in coping with stressors both behaviorally (Wingfield & Romero, 2001) and physiologically (Dallman *et al.*, 1993; Sapolsky *et al.*, 2000). However, long-term glucocorticoid exposure can be devastating. These hormones can cause a multitude of deleterious effects, including shutting down reproduction and the immune system (Sapolsky *et al.*, 2000), and even causing neuron death (Sapolsky, 1992).

Work from the past 20 years has examined glucocorticoid release in wild animals (Wingfield & Romero, 2001). Plasma levels of glucocorticoids can be used as a measure of 'stress' (i.e. health) in wild animal populations (Wingfield *et al.*, 1997). Stressors that have been examined include several natural stressors such as weather conditions affecting birds (e.g. Smith *et al.*, 1994; Romero *et al.*, 2000) and social interactions in social species such as wild dogs (Creel *et al.*, 1996) and baboons (Sapolsky, 1987). Several studies have also examined human created stressors. Examples include deforestation of spotted owl habitat (*Strix occidentalis*, Wasser *et al.*, 1997), heavy metal contamination in trout (*Salmo trutta*, Norris *et al.*, 1997), and coal waste pollution in ponds supporting toad populations (*Bufo terrestris*, Hopkins *et al.*, 1997). All of these stressors have resulted in increased glucocorticoid levels in the affected species. On the other hand, some human disturbances do not appear to result in elevated glucocorticoid levels. Tourist visitation of animal breeding grounds, especially common with increases in ecotourism, does not appear to stimulate glucocorticoid release in either magellanic penguins (*Spheniscus magellanicus*, Fowler, 1999), or Galápagos marine iguanas (Romero & Wikelski, in press), but does so in a Neotropical bird, the Hoatzin (*Opisthocomus hoazin*; Müllner, Linsenmair and Wikelski, in preparation).

Galpagos Marine Iguanas: A Case Study

Our recent work has focused on understanding the stressors that impact wildlife, especially the Galápagos marine iguanas as a model system for a previously undisturbed (by humans) animal species. Galápagos marine iguanas offer an ideal model system because island populations are affected differently by recurring El Niño famines. El Niño is a recurring global climate event whose main impact in the eastern Pacific is a change in trade winds and the subsequent failure of nutrient-rich upwelling (Martin *et al.*, 1993). Marine iguana populations can decline by as much as 90% during severe El Niños (Laurie, 1989; Wikelski & Trillmich, 1997).

During 1997 and 1998 one of the longest and most severe El Niños on record struck the Galápagos Islands. Water temperatures in the Galápagos Archipelago, normally between 18 and 23 °C, remained elevated up to 32 °C for nearly 18 months (Oberhuber *et al.*, 1998). This led to a severe reduction in the algal forage of the marine iguanas and resulted in widespread starvation, as observed during previous El Niños (Laurie, 1989; Wikelski & Trillmich, 1997). We examined the levels of corticosterone (the primary glucocorticoid in reptiles, Greenberg & Wingfield, 1987) during and after this major El Niño event. Because it generally takes several minutes after the initiation of a stressor before elevated corticosterone is detectable (Sapolsky *et al.*, 2000), we were able to measure both baseline corticosterone (reflecting samples taken immediately after capture and assumed to represent levels prior to the stress of capture) and stress-induced corticosterone (measured 15–30 min after capture).

We captured marine iguanas from six islands in the Galápagos within one week before widespread nutrient upwelling ended the 1998 El Niño conditions (Fig. 1).

Many animals were in extremely poor condition and carcasses were abundant. We also sampled animals exactly one year after the El Niño event, during a 'normal' year. Corticosterone levels were generally low during the 1999 La Niña feast period, but the 1998 El Niño famine resulted in both higher baseline and capture-stress induced corticosterone concentrations (Romero & Wikelski, 2001). Corticosterone levels were

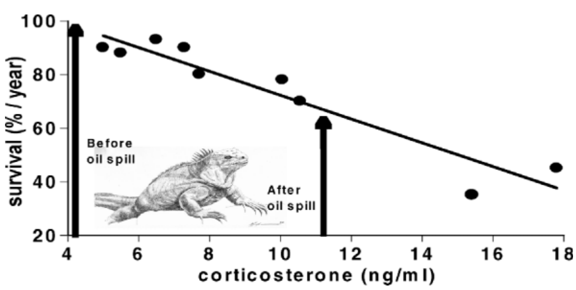

Fig. 1 Marine iguanas show a stress response to capture and handling by elevating corticosterone in their blood plasma. We captured animals on six different islands during 1999 and during the El Niño of 1998. Each data point represents the mean ± SE for one island in one year. The linear regression line indicates the predicted survival rate based on our population counts. The vertical arrows show the corticosterone levels of marine iguanas before and after an oil spill hit Santa Fe island, one of our long-term study sites (redrawn after Romero & Wikelski, 2000).

higher during El Niño, even though absolute corticosterone levels differed between islands.

Individuals with high corticosterone levels had a poor body condition, i.e. starved. Corticosterone is elevated during starvation in many species (e.g. Jeffrey *et al.*, 1985; Fichter & Pirke, 1986; Young *et al.*, 1987), and since high corticosterone levels are known to promote protein catabolism (Dallman *et al.*, 1993), these data suggest that corticosterone may have been helping mobilize protein reserves as a last-ditch effort to try to survive.

Perhaps the most exciting find from this study is that corticosterone levels on the different islands predicted survival through the El Niño period. The severity of the El Niño differed on each island (due to different ocean currents, etc.), resulting in different levels of survival on each island. These survival estimates, however, were highly correlated with the mean stress-induced corticosterone levels for the respective island (Romero & Wikelski, 2001). Thus, even though the corticosterone response varied at the population level (i.e. each island had a different corticosterone response), nevertheless, stress-induced corticosterone levels predicted overall population health.

A Low-Level Oil Spill: High Wildlife Mortality Confirming the Stress Predictions

The ability to predict survival turned out to have an immediate application. On January 17, 2001, the oil tanker "Jessica" ran ashore on San Cristobal Island (Fig. 2a). Within a week, roughly three quarter million gallons of diesel and bunker oil spilled throughout the southwestern islands of the archipelago (Fig. 2b).

We had fortuitously collected blood samples 3 days before the oil spill as part of the El Niño study, and repeated the sampling 7 days after the spill on the same population of animals (Fig. 3; Wikelski *et al.*, 2001).

Few animals died immediately from the physical impact of oiling, even though oil patches were visible in tide pools throughout the study area and 70% of individuals had oil residues on their skin. We determined both baseline and stress-induced corticosterone levels and compared the post-spill data to the pre-spill data, and found that both were substantially higher after the spill (Wikelski *et al.*, 2001). Furthermore, animals with and without visible external oil blotches did not differ which may indicate either that oil residues were ingested while feeding in polluted intertidal areas or that animals had decided not to forage because of the fouled grazing areas (Fig. 3).

We then used the corticosterone-to-survival relationship, as determined during the El Niño, to try to predict survival of the oiled iguanas (Fig. 1). The pre-oil spill corticosterone concentrations predicted an annual survival rate of more than 95%, typical of normal survival rates during good conditions (Laurie, 1989; Wikelski *et al.*, 1997), but the post-oil spill corticosterone concentrations predicted iguana mortality rates of about 40%.

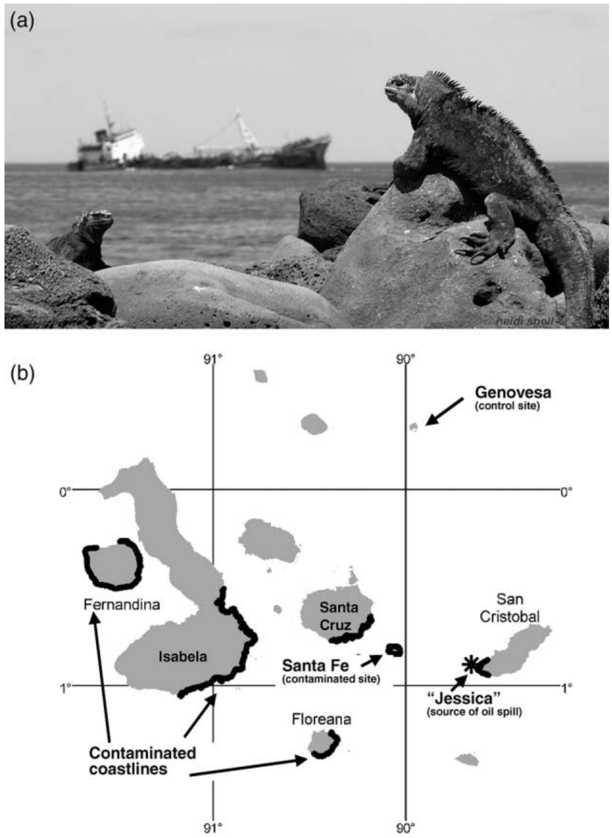

Fig. 2 (a) The tanker "Jessica", grounded a few hundred meters offshore from San Cristobal Island, Galápagos, Ecuador. A few days after the spill the hull broke, spilling several hundred thousand liters of bunker oil and diesel into the Galápagos Natural World Heritage site (© Heidi Snell). (b) Map of the Galápagos archipelago indicating the site of the spillage, our two study sites (control: Genovesa; oiled: Santa Fe) and the approximate distribution of oil contamination along the shorelines as determined by coastal survey through the Galápagos National Park and the Charles Darwin Research Station (redrawn after Wikelski *et al.*, 2002).

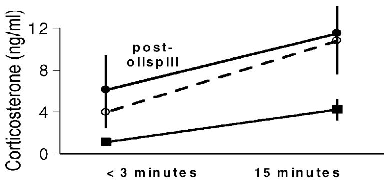

Fig. 3 Corticosterone levels of Santa Fe marine iguanas shortly before and shortly after exposure to trace oil pollution. Iguanas had low levels of both baseline and stress-induced levels before the spill. After the spill, both externally visibly contaminated (solid points and line) and externally clean (dotted line) iguanas had elevated corticosterone levels (redrawn from Wikelski *et al.*, 2001).

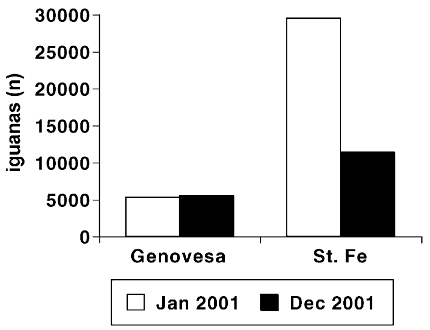

Fig. 4 Population counts of marine iguanas on Genovesa (control island) and Santa Fe (oil contaminated island) before (January 2001) and about 1 year after (December 2001) the Jessica oil spill. The estimated total Santa Fe iguana population declined dramatically, while the Genovesa control population was stable (redrawn after Wikelski *et al.*, 2002).

Actual mortality was somewhat higher than the predicted 40%. We returned to the study site approximately one year later and found 62% had died (Fig. 4; Wikelski *et al.*, 2002).

This mortality was also qualitatively different from mortality due to El Niño conditions (Fig. 5a and b).

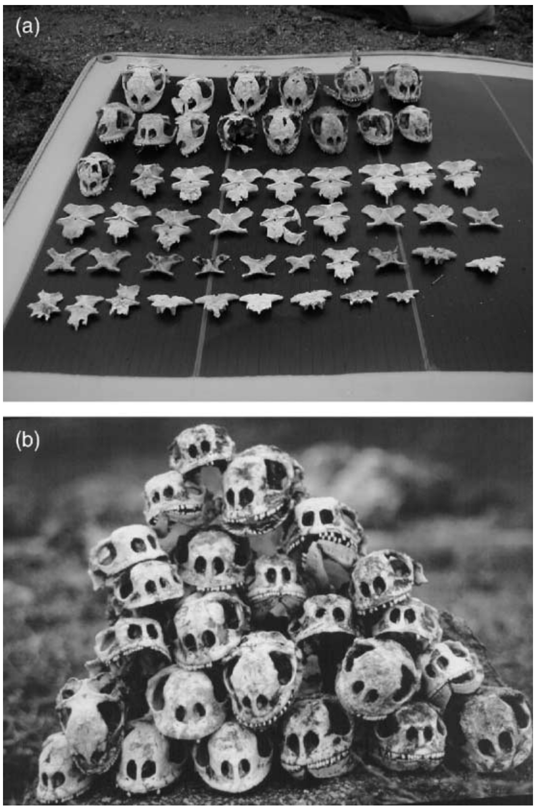

Fig. 5 (a) Selection of skulls of marine iguanas that died in the aftermath of the Jessica oil spill on Santa Fe island. Note that both large and small brain capsules were found along a 100 meter stretch of coastline. (b) Contrary to Fig. 5a, all marine iguanas that died during the 1997/98 El Niño event were large iguanas, as indicated by the uniformly large skulls found in the aftermath along the same stretch of coastline.

An El Niño has a disproportionate effect on the largest individuals in the population, such that these animals are the most likely to die (Laurie, 1989; Wikelski *et al.*, 1997). The oil spill, on the other hand, killed both younger and older animals in equal proportions (Fig. 5a and b; and Wikelski, unpublished data). The most important result of this work, however, is that the corticosterone levels in the oiled iguanas predicted mortality that was not apparent immediately after the spill. The lack of dead animals immediately after the spill lead the media and other observers to conclude that the spill had not been very bad, but the corticosterone levels predicted the final reality that the oil spill had a devastating impact on the marine iguanas.

However, it is still unclear why exactly the iguanas died in such large numbers. We hypothesize that the iguana's hindgut bacteria, which digest the algae cellulose (Mackie *et al.*, submitted for publication), were wiped out by trace oil contaminations. Thus, marine iguanas still foraged normally and appeared fine, but could not actually digest the food. A lack of nutrients in the blood then caused a rise in corticosterone, which mobilized internal resources and finally starved the affected animals to death.

Further Research Needs

We suggest that future research should address the following main questions:

- How are wildlife populations in diverse habitats affected by trace oil contaminations?
- What is the exact chain of causation that kills animals after low-level oil contamination, and is corticosterone playing a direct role?
- How good a predictor is the corticosterone stress response for survival in other animal populations?

In particular, we suggest that the Galápagos marine iguanas could be continued to be monitored in the aftermath of the oil spill. Specific research needs are:

- How exactly are hindgut bacteria affected by trace oil?
- Can reinoculation of bacteria from healthy animals remedy a loss of bacteria in affected individuals?
- Can corticosterone levels be used to predict the effectiveness of the oil remediation and cleanup)?
- How does corticosterone affect starvation and tissue mobilization?
- Are high corticosterone levels related to the observed shrinkage of body length in marine iguanas (Wikelski & Thom, 2000)?

The ideal solution to address these and a variety of additional research needs is to start a long-term, internationally funded ecological monitoring program in the Galápagos Islands, perhaps as part of NSF's long-term ecological research (LTER) program.

Summary and Conclusions

The set of studies on the stress response in Galápagos marine iguanas described here has played an important role in understanding how human stressors can impact wild animals. They have provided a tool by which researchers can estimate the impact of a stressor, such as an oil spill, without waiting for the bodies to be counted. Elevated corticosterone levels can be a rapid indication that the health of a population has been affected, and suggest that ameliorative actions should be initiated. The important research questions arising from our findings could best be addressed in a long-term ecological research setting.

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