Can we distinguish between the speed and scope of acute glucocorticoid responses?

Conor C. Taff1,2\*, Laura Schoenle3, Maren N. Vitousek1,2

1 Department of Ecology & Evolutionary Biology, Cornell University

2 Lab of Ornithology, Cornell University

3 ??

\* Correspondence: Conor C. Taff, [cct63@cornell.edu](mailto:cct63@cornell.edu) 518-332-3983

Notes:

* Helped with field work (my memory is kind of fuzzy here, I’m not sure this is really right and I might be missing or adding some of the wrong people…): Tom, David, Natalie, ???

**ABSTRACT**

***Keywords:***

**INTRODUCTION**

1. Glucocorticoid response critical part of the system that allows animals to cope with challenging and often unpredictable situations: emergency life history stage.
2. Long and productive history of research beginning in 70s/80s on cort in wild animals that established methods for measurement
   1. To a large extent, this early work still defines the way that cort is measured: baseline sample <3 min and induced sample at 15/30/60 minutes with that time point being largely determined by early studies that determined when a typical species reached its maximal cort level
3. Early research largely focused on two questions:
   1. comparing groups of individuals in different conditions (breeding vs. non, storm vs good conditions, migration vs. not, well fed vs. hungry).
   2. Comparing different species or populations that have different environmental experiences (latitudes, predictability, etc).
4. Starting around ??? (Fran’s 2009 paper? Earlier?) a big focus of cort related work shifted to trying to understand whether individuals in a population differed in their base/induced cort levels and whether those differences were related to important life history characteristics (coping ability, reproductive success, etc).
5. Even more recently, there has been a huge amount of interest in understanding between individual differences in the flexible adjustment of cort within a single acute response or between different contexts.
   1. These development of questions in this field has thus moved from group averages, to individual differences, to understanding the actual shape of the acute response as a time series
   2. Despite the changing goals of research questions, the general measurement approach of base + stress sample has largely remained unchanged for most studies.
6. Here, we focus on one particular question about the shape of the acute stress response: speed vs scope (Taff & Vitousek), but the general approach that we take is applicable to many current questions that seek to apply reaction norm approaches to cort data.
   1. The question boils down to: can we effectively estimate the shape of a cort response from two time point samples at 0 + 30 minutes?
   2. The answer to this question will likely depend on a variety of aspects of the stress response that have not been explored in most study systems: are the speed and scope tightly correlated, such that fast responders are also high responders? Are the shape and absolute level of cort responses more or less repeatable?
   3. We approach this problem in two steps, first we explore a field data set in which we measured the corticosterone response to acute handling stress in RWBL using a 7 point time series that allows us to more finely measure in different aspects of the time course of a stress series than typical studies that use only 2-3 time points (this dataset also includes breeding and non-breeding season measures and we explore seasonal differences).
   4. Second, we develop a simple simulation that generates a cort time series for a set of animals with desired characteristics. This simulation allows us to generates datasets with varying degrees of correlation between different features of the cort response and ask whether sampling schemes typically employed in field studies can recover the effects present in the data generating process. The simulation can also be used to create simulated datasets with desired characteristics that researchers can use to determine whether their study design is capable of detecting the effects they wish to study. Our simulation is very simple and is not meant to capture the mechanistic details of a cort response, but it provides a starting point for evaluating whether the methods currently employed in this field provide a viable route to answering the questions being addressed.

**METHODS**

*Data Collection*

*Data Analysis*

*Description of Simulation*

We developed a set of functions in R that produce simulated glucocorticoid response datasets with desired characteristics. We note that this simple simulation is not intended to be a true mechanistic representation of the glucocorticoid stress response or to capture all of the kinematic reality of glucocorticoid regulation. Rather, our simulation produces curves that are similar in shape to real glucocorticoid responses, but where causal links between curve attributes and performance are known and where parameters can be easily varied to mimic possible real-world patterns.

We began by creating an initial function that samples a set of underlying parameters for a population of animals of a set size. Because the data are simulated, the units are arbitrary, but we set value ranges and refer to parameters in units that are similar to those typically observed and reported in wild animals for convenience. The parameters that we defined were baseline glucocorticoid level (ng/μl), speed of the acute response (minutes to reach maximum), maximal glucocorticoid level (ng/μl), time spent at the maximal level before beginning to decline (duration in minutes), and time of return to baseline levels (minutes from start). The function that we wrote allows us to set the mean and standard deviation of each parameter independently. For maximal glucocorticoid level, we sample from values on a log scale before back-transforming to capture the skew observed in real world datasets; all other parameters are sampled from a normal distribution.

In addition to setting the mean and standard deviation for each individual parameter, the function allows specification of covariance structure between each pair of parameters. After setting these values, we used the ‘mvrnorm’ function in the ‘MASS’ package in R to sample parameters for each animal in the population (CITE). We consider these parameters to be the ‘true’ phenotypic values of the individual (i.e., in a real study these values would be unmeasured). The ‘true’ phenotypic values are used to generate a single performance measure for each animal by defining the relative contribution of each of the five parameters and of random variation to performance. This performance measure can be thought of as fitness or some fitness proxy but is presented as a unitless performance measure here.

We next wrote a second function that takes as input the dataset produced by and generates a set number of samples for each animal based on their underlying phenotypic values. To generate these samples, we add a normal random error to each of the five parameters described above for each sample; the amount of error can be specified separately for each parameter. This noise can be thought of as measurement error or—when generating multiple samples per individual—as within-individual variation in glucocorticoid expression plus measurement error. Using the five parameter values sampled with noise, we next fit a loess smoothed regression for each sample and then determine the corticosterone value at each minute during the entire time-course of the stress response. The function returns a list that includes the full dataset with a measure for each time point, a down-sampled dataset with measures at defined time points (by default at 2 and 30 minutes as typical for many field studies), and a rank transformed dataset that is useful for visualizing how the rank order of glucocorticoid levels changes over the time-course among individuals. This output can be transferred to any number of other modeling or plotting packages in R depending on the question being addressed, but we also provide a simple plotting function that creates a summary plot of the simulation quick visualization.

Together, these two functions can produce with one line of code a realistic simulated dataset containing a full time-course of glucocorticoid curves sampled with modifiable error for an arbitrary number of animals and an arbitrary number of captures per animal along with a performance outcome that is linked to the underlying phenotype. The full set of functions along with a brief tutorial are available on GitHub (<https://github.com/cct663/speed_vs_scope>). Below, we use the functions to explore several scenarios pertaining to differentiating the speed versus scope of glucocorticoid responses, but we hope the tool will also be useful for researchers interested in other scenarios not covered here and for generating artificial datasets that can be used to ask whether a planned sampling design has the ability, in principle, to answer a particular research question. Additionally, while the simulation is written with glucocorticoid responses in mind, it should be equally applicable with minor modifications to other function valued traits in which the research question is about the shape of a response curve, but sampling must be done at discrete time points.

*Simulation Parameter Exploration*

**RESULTS**

*Field Study*

*Simulation Results*

**DISCUSSION**

* What we really need: ability to measure timecourses of cort responses continuously or at least at a finer temporal resolution than 2-3 points over the course of an hour.
  + Solution: has to be some combination of
    - measuring more animals at different time points (e.g., clones/relatives/selected lines that are measured at different times and put together to reconstruct overall response shape)
    - non-invasive measurement techniques that allow for finer sampling: biologgers, fluorescent readers, proxies like HR – all need to be validated
* These solutions also raise another major problem that plagues this field: we are interested in measuring the time course of an event (the acute stress response) but our continued handling and sampling of animals influences that very process. A ‘real’ cort response might often involve a very short stimulus that is gone quickly but the hormone lingers, but in a capture restraint protocol, would cort ever return to ‘baseline’ levels?? How to our measures relate to the actual time course of these events?
* Call for more attention to method development and validation of measurement in field studies. Early studies in the 70s & 80s spent a lot of focus on this, and the methods that they developed were very powerful for the types of questions being addressed. It isn’t as clear that the methods used today are as well validated or matched to the questions of focus. Rather than just adopting 0 + 30 sampling, more work is needed in different species to understand the shape and amount of variation in timing in order to make progress on questions relating to the reaction norms of cort phenotypes.
* Methods exist for analysis: function valued traits, reaction norm approaches, etc. The limitation here is really the availability of field data. In the meantime, much progress could be made by simulations that use real parameters derived from populations (more sophisticated than what we present here) to generate testable predictions. Luttbeg + Grindstaff paper is one example (is that published yet?). These approaches can also help to define optimized sampling schemes.

**ETHICAL NOTE**

**ACKNOWLEDGEMENTS**

**FUNDING**

**REFERENCES**