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Baseline Corticosterone in Wintering Marine Birds: Methodological Considerations and Ecological Patterns

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ever, most studies relating plasma CORT in seabirds to foraging conditions have focused on the breeding period (Romero 2002), and it is unclear whether similar relationships between foraging conditions and baseline CORT exist during other life stages.

Measuring blood plasma levels of baseline CORT in the field is challenging because capture and bleeding are stressors that can result in acute stress responses (Romero and Romero 2002). The relationship between time since capture and acute CORT response has been well documented, and it is clear that CORT levels rise within minutes of exposure to stressors; thus, accurate assessment of baseline CORT requires that time between capture and bleeding be below the point at which acute CORT responses are detected (Wingfield et al. 1982; Romero and Romero 2002; Romero and Reed 2005).

In some species (e.g., open-water seabirds) or during particular stages of the annual cycle, lethal sampling may be the only viable means to collect blood samples. Such sampling eliminates stress associated with capture and handling, but approaching or pursuing birds also may result in acute stress. However, the few studies that have published CORT data from lethally collected birds have not assessed changes in CORT levels as a function of the time between initial disturbance and death (Lisano et al. 1977; Whatley et al. 1977; Wingfield et al. 1982; Marra et al. 1995).

White-winged scoters (*Melanitta fusca*) are sea ducks that spend most of the year in marine habitats. Although the British Columbia (BC) coastline entails a significant portion of their wintering range on the Pacific coast of North America, there are few wintering areas in BC where white-winged scoters occur in high concentrations (J.-P. L. Savard, unpublished data). This may be due in part to their preference for soft-bottom habitats with abundant bivalve foods, which are relatively uncommon in BC. However, even among such sites, there is considerable variation in habitat conditions that may affect baseline CORT levels of white-winged scoters. Widespread population declines in white-winged scoters and other sea duck species have prompted efforts to identify functional roles of different habitats used throughout the annual cycle by analyzing body condition, prey availability, foraging effort, diet composition, and plasma metabolite analyses (e.g., Zydulis et al. 2006; Anderson and Lovvorn 2008, 2011; Anderson et al. 2008; Lewis et al. 2008; Palm et al. 2012). Baseline CORT could complement

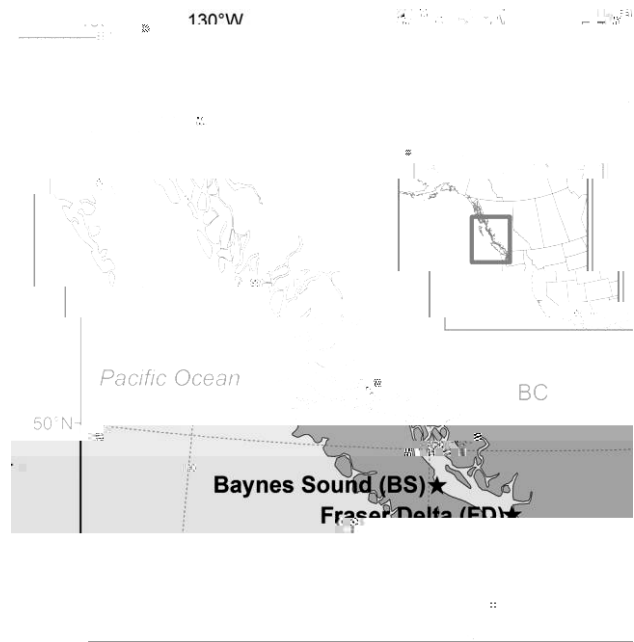


Figure 1. Map of four study sites along coastal British Columbia where studies of plasma CORT in wintering white-winged scoters were conducted during 2009–2011.

collected only after-hatch-year (i.e., mature/adult) males as verified by plumage (Brown and Fredrickson 1997). We recorded the time since flush for each bird, which we defined as the period between initial flush and death. Because of the difficulty of determining when a bird first began to respond to our presence on our approach, times since flush were estimated and rounded to the nearest whole minute. We generally approached small flocks at speeds of 15–25 knots and shot flying birds from a moving boat. We made all efforts to ensure that all birds were killed cleanly and quickly. Within 5 min of retrieval, we sampled heart blood using a sterile 18-gauge needle and transferred up to 5 mL of blood to a heparinized vial for CORT analyses. We centrifuged whole blood within 8 h of collection to separate plasma and cells and stored all tissues at -20°C . To facilitate examination of relationships between CORT and metrics of individual condition, Long Point Waterfowl's Avian Energetics Lab (Port Rowan, Ontario) conducted analyses of body composition (total protein, lipid, water, and ash) from ingesta-free dry carcass mass, including plumage, following methods of Afton and Ankney (1991).

CORT Assay

We determined concentration of total CORT in nonextracted plasma using a corticosterone enzyme-linked immunoabsorbent assay (EIA-Assay Designs, catalog no. 901-097) with a four-parameter logistic fit based on Love and Williams (2008). We ran all samples in triplicate across six assay plates at a total volume of 100 μL with 1:40 dilution and 1.5% steroid displacement buffer. As per kit instructions, we first incubated plates at 26°C under shaking at 500 rpm for 2 h and then at 26°C without shaking for 1 h; we calculated the detection limit of the assay at 0.018 ng/well (0.72 ng/mL), with intra- and interassay coefficients of variation of 6.89% and 10.15%, respectively.

Study Sites

Each study site (Fig. 1) represented a different combination of three habitat conditions, which we assessed for their influence on baseline CORT: exposure to wind and waves, water depth, and predation danger (table 1). Along the Pacific Coast, many white-winged scoters winter in somewhat protected nearshore areas. Dog Sh Banks (53°55'N, 131°30'W) is a notable exception because it is an offshore site that is particularly susceptible to

turbulent seas during winter storms due to frequent high winds from the southeast (RPS Energy 2009). Combined with a mobile substrate of sand or a sand-gravel mixture, these conditions result in a frequently changing benthic foraging habitat. At Dog Sh Banks, there is virtually no presence of bald eagles (*Haliaeetus leucocephalus*), which are the most likely predator of white-winged scoters (Anderson et al. 2012). Relative to other study sites, Dog Sh Banks birds feed on a more diverse suite of prey (Palm et al. 2012). Water depths at the study site range from 4 to 20 m, and the maximal tidal range is approximately 5 m (Amos et al. 1995).

Situated among many large islands and the mainland coast, Chatham Sound (54°27'N, 130°25'W) is more protected from high winds and large swells than Dog Sh Banks. Birds at Chatham Sound feed in deep water (5–30 m), searching for prey in sandy and muddy substrate situated between rock outcrops (E. Palm, personal observation). Bivalves are the main prey taken by white-winged scoters at this site, but during some periods, birds at Chatham Sound consume a wider variety of foods, including echinoderms and crustaceans (Palm et al. 2012).

Baynes Sound (49°39'N, 124°53'W) contains extensive intertidal flats and high densities of wild and cultured bivalves, providing nearshore habitat for about 6,500 white-winged and surf scoters during winter (W. S. Boyd, unpublished data). Because it provides an abundant and temporally stable source of bivalve prey and white-winged scoters wintering in the area show relatively low foraging effort, Baynes Sound is thought to be high-quality foraging habitat (Lewis et al. 2007, 2008). Unlike on Dog Sh Banks, white-winged scoters wintering on Baynes Sound and Chatham Sound often forage in close proximity to high densities of bald eagles.

Thousands of white-winged scoters use the shallow intertidal mud flats on the Fraser River Delta (49°06'N, 123°16'W) annually for molting, wintering, and staging (J. R. Evenson, unpublished data). The Fraser River Delta offers very few potential perches for bald eagles. Similar to scoter diets in Baynes Sound, diets on the Fraser River Delta consist almost entirely of bivalve prey (Palm et al. 2012).

Data Analysis

Quantification of Time Line to Acute Stress. Before running the models corresponding to each of our a priori hypotheses describing possible sources of variation in baseline CORT, we

Table 1: Variation in habitat conditions and diet composition among four wintering sites for white-winged scoters in British Columbia

Study site	Exposure	Water depth	Proportion of bivalves in diet	Predation danger
Chatham Sound	Low	High	High to very high	High
Dog Sh Banks	High	High	Medium to high	Low
Baynes Sound	Low	Low	Very high	High
Fraser River Delta	Low	Low	Very high	Low

quantified the time between initial flush of targeted birds and acute stress response, represented by an increase in plasma CORT concentration. We excluded a single data point from all analyses, with a CORT concentration of 151.3 ng/mL and an associated time since flush of 1 min, because it far exceeded baseline values found in a separate study of wintering sea ducks, which ranged from approximately 15 to 50 ng/mL (Nilsson et al. 2008). An initial plot of CORT versus time since flush showed average CORT to be relatively stable until approximately 3–4 min, after which it increased (Fig. 2). We ran a series of multiple linear regressions in R (R Development Core Team 2011) to determine the appropriate cutoff time that would separate baseline values from values of potentially acutely stressed birds (Nickerson et al. 1989).

Using 1 min as the first potential cutoff time, we separated CORT data into two groups based on associated time since flush: (1) 0–1 min and (2) 1–11 min. For each of the two groups in the linear model, we allowed both the intercept and the slope to vary. We then repeated the process using every time since flush from 2 to 6 min as the potential cutoff time between the two groups. We expected to see a relatively flat line for baseline values, followed by a linear increase in CORT over time, which would represent acute stress. To choose the cutoff time that provided the best fits of both baseline CORT and the acute stress response, we calculated Akaike's Information Criterion adjusted for small sample size (AIC_c) for each of the models and chose the model with the lowest value (Burnham and Anderson 2002). We log transformed CORT data to meet assumptions of normality in our linear models, but we report untransformed data.

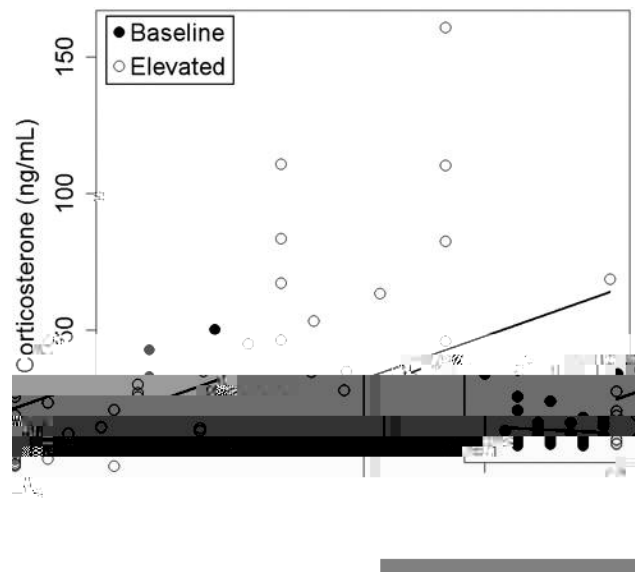


Figure 2. Plasma CORT concentrations as a function of time since flush (i.e., the period between initial flush and death). Separate regression lines for baseline levels (0–3 min) and elevated levels (1–13 min) were derived from the best-supported model evaluating appropriate cutoff time.

Table 3: Post hoc candidate models describing variation in baseline levels of plasma CORT in male white-winged scoters across four wintering areas in British Columbia

Explanatory variable	K	DAIC _c	w	r ²
Protein mass	4	.00	.89	.18
Body mass	4	5.02	.07	.11
Time-of-day covariate only	3	7.86	.02	.04
Lipid mass	4	8.33	.01	.07
Null	2	8.68	.01	...

Note. Results account for inclusion of a covariate for time of day for all models except the null.

ush until death is measured. We found increasing CORT values above a time since flush of 3 min, presumably reflecting acute stress responses. That result is consistent with past studies indicating that blood samples taken within 3 min of capture represented baseline CORT (Wingfeld et al. 1982; Romero et al. 1997; Silverin and Wingfeld 1998; Romero and Romero 2002; Romero and Reed 2005). Our methods, which determined the appropriate cutoff time between baseline and stressed concentrations of CORT in shot birds, can be applied in subsequent studies requiring lethal sampling of birds.

Despite differences in habitat conditions, baseline CORT of white-winged scoters did not differ across sites. These results suggest three possibilities: (1) overall foraging conditions were

prey base support fewer larger animals? *J Anim Ecol* 78:1033-1042.

Reneerkens J., M. Ramenofsky, and T. Piersma. 2002. An experimental test of the relationship between temporal variability of feeding opportunities and baseline levels of cor-