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Effects of ACTH, capture, and short term confinement on glucocorticoid concentrations in harlequin ducks (*Histrionicus histrionicus*)

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Abstract

Little is known about baseline concentrations of adrenal hormones and hormonal responses to stress in sea ducks, although significant population declines documented in several species suggest that sea ducks are exposed to increased levels of environmental stress. Such declines have been observed in geographically distinct harlequin duck populations. We performed an adrenocorticotropic hormone (ACTH) challenge to evaluate adrenal function and characterize corticosterone concentrations in captive harlequin ducks and investigated the effects of capture, surgery, and short term confinement on corticosterone concentrations in wild harlequin ducks. Harlequin ducks responded to the ACTH challenge with an average three-fold increase in serum corticosterone concentration approximately 90 min post injection, and a four- to five-fold increase in fecal glucocorticoid concentration 2 to 4 h post injection. Serum corticosterone concentrations in wild harlequin ducks increased within min of capture and elevated levels were found for several hours post capture, indicating that surgery and confinement maintain elevated corticosterone concentrations in this species. Mean corticosterone concentrations in wild harlequin ducks held in temporary captivity were similar to the maximum response levels during the ACTH challenge in captive birds. However, large variation among individuals was observed in responses of wild birds, and we found additional evidence suggesting that corticosterone responses varied between hatch year and after hatch year birds.

Keywords: ACTH; Adrenal function; Corticosterone; Glucocorticoids; Harlequin duck; Sea ducks; Stress

1. Introduction

The glucocorticoid (GC) hormones, cortisol and corticosterone (CORT), are secreted by the adrenal cortex and play important roles in gluconeogenesis, mobilization of energy, and growth and development in birds (Cahill, 1971; Thompson and Lippman, 1974; Wingfield et al., 1994; Norris, 1997). GCs also influence many other physiological processes that allow an animal to respond and function in situations that it perceives as stressful and thus GCs are also referred to as stress hormones (Harvey et al., 1984). CORT has been established as the primary circulatory GC in birds (Holmes and Phillips, 1976; Harvey and Phillips, 1982; Wingfield et al., 1992). Several factors have been found to increase circulating CORT concentrations in birds, including insufficient food and water (Rees et al., 1985; Harvey and Hall, 1990; Kontecka et al., 1999; Kitaysky et al., 2001), capture and handling (Wingfield et al., 1982; Romero et al., 1997; Gratto-Trevor et al., 1991; Silverin and Wingfield, 1998), social rank (Schwabl et al., 1988; Nunez-De La Mora et al., 1996), inclement weather (Smith et al., 1994; Romero et al., 2000), petroleum contamination (Holmes et al., 1979; Fowler et al., 1995), and heat stress (Edens and Siegel, 1975). A

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temporary increase in circulating CORT concentrations allows a shift in energy allocation from physiological and behavioral processes that are non-essential for immediate survival to those that require rapid response to an environmental change or stressor (Kacsoh, 2000; St. Aubin, 2001). The physiological and behavioral effects of a short term increase in CORT concentrations (i.e., "rapid" or "acute" stress response) are considered adaptive and beneficial, whereas long-term exposure to elevated GC concentrations (i.e., "chronic" stress) can be devastating to an organism (Wingfield et al., 1998). In birds, effects of chronic or prolonged increases in circulating GCs include reduction in size of the ovary (Petite and Etches, 1991) and testes (Gross et al., 1980), gastrointestinal dysfunction (Monnikes et al., 1994), growth inhibition (Davison et al., 1980), suppression of the immune response (Gross et al., 1980; Wingfield et al., 1998), and eventually death (Wingfield et al., 1998).

The media most commonly used to assess CORT concentrations in birds are plasma and serum (Wingfield et al., 1982; Silverin et al., 1993; Wingfield et al., 1994). Because capturing of wild birds has been shown to constitute a stressor, it is imperative that blood samples be obtained rapidly after capture in order to get a baseline concentration of CORT (Wingfield et al., 1982; Romero et al., 1997; Silverin and Wingfield, 1998). Several studies have shown that other media (such as feces and cloacal fluids) also can be used to quantify GC concentrations (Wasser et al., 1997; Hiebert et al., 2000; Wasser et al., 2000; Goymann et al., 2002). When measuring fecal adrenal products, the use of the term "glucocorticoid metabolites" (GCM) has been recommended (Möstl et al., 2005; Touma and Palme, 2005). Using feces or cloacal fluids for GCM analysis has the advantage of being less invasive (or non-invasive in cases when feces are obtained without capturing and handling the bird) than using serum (Wasser et al., 2000). Furthermore, elevated GCM concentrations in response to a stressor typically occur in feces on the order of hours rather than minutes after the stressor. Fecal samples may be used to investigate baseline concentrations of GCM when it is difficult or impossible to obtain a blood sample within a few minutes of capture (Hiebert et al., 2000; Goymann et al., 2002.; Millspaugh and Washburn, 2003). Injection of exogenous adrenocorticotropic hormone (ACTH) can be used to stimulate a physiological stress response characterized by elevated GC concentrations. This procedure allows researchers to identify the potential magnitude of the stress response as well as the lag-time between the stressor (e.g. ACTH) and the maximum response. ACTH has been used to evaluate adrenal function in several avian taxa (e.g., psittacines: Zenoble et al., 1985a; raptors: Zenoble et al., 1985b; waterfowl: Spelman et al., 1995; and cranes: Ludders et al., 1998).

The harlequin duck (*Histrionicus histrionicus*) is a member of the tribe Mergini, or sea ducks, which consists of 18 species typically occurring at high latitudes in the northern hemisphere. Several members of this group have undergone considerable declines in recent decades and the causes are unclear for most species (Stehn et al., 1993; Henny et al., 1995; Suydam et al., 2000; Sea Duck Joint Venture Management Board, 2001). Sea ducks may be especially sensitive to anthropogenic disturbances (e.g., hunting, contamination, and loss of habitat) and as these become more prevalent in their natural environment, further population declines may occur (Henny et al., 1995; Irons et al., 2000; Wentworth and Wong, 2001). Few studies have investigated stress and GC concentrations in this group of birds (Perfito et al., 2002; Wayland et al., 2002; Wayland et al., 2003), nor has the adrenal reaction characterized by maximum CORT output in response to an ACTH challenge been documented for any sea duck species. The adrenal response to an ACTH challenge may help define acute and chronic stress in this species by characterizing the magnitude and shape of the stress response curve, and could aid in identifying populations under chronic stress. Quantifying basic physiological parameters in species of conservation concern is important for establishing tools that can be used to evaluate population constraints. Understanding adrenal function in sea ducks will enhance our knowledge of the physiology of these species and may ultimately help elucidate the relationship between these birds and their environment by investigating the interaction of environmental stressors and sea duck populations under stress.

The objectives of the present study were to characterize adrenal function in harlequin ducks by: (1) validating the use of a commercially available radioimmunoassay kit to evaluate GC concentrations in serum and feces, (2) performing an ACTH challenge to investigate the lag-time between exposure to a stressor (exogenous ACTH), the peak GC concentration, and the duration of the stress response in captive ducks, and (3) investigating the effect of capture and holding on serum GC concentrations in free-ranging individuals.

2. Materials and methods

2.1. Captive harlequin ducks

Female harlequin ducks for captive studies ($n=10$) were caught in Prince William Sound (PWS), Alaska (60° 45′ N; 156° 47′ W), in September 2001 during wing molt (Rizzolo, 2004). These birds were subjected to low dose oil ingestion and plumage oiling experiments between October 2001 and February 2002 (Rizzolo, 2004). At the start of the present study (late April 2002) the birds were healthy and no difference in CORT concentrations could be detected between the ducks which had been subjected to external/internal oiling experiments and un-oiled ducks in the control group. The ducks were housed in outdoor enclosures under ambient conditions at the Alaska SeaLife Center, Seward, Alaska (60° 69′ N; 149° 26′ W). Each enclosure had a floor space of 8.7 m^2 and a circular saltwater pool with an area of 4.7 m^2. The enclosures each housed five birds. The average mass of the birds used in this study was 533 g (range: 492 to 613 g).

2.2. Sample collection

Blood samples were drawn via jugular, brachial, or tarsal venipuncture utilizing a sterile 1 cc syringe and a 25 gauge needle. All samples were stored at 4 °C prior to processing. Blood was centrifuged at 1,500 g for 10 min using a Clay Adams® TRIAC® centrifuge (Becton Dickinson Company,

Franklin Lakes, NJ). Serum was harvested from the samples and stored at −80 °C until analysis.

Fecal samples were obtained opportunistically from a sheet of aluminum foil placed on the bottom of individual transport kennels used to hold individual birds during the ACTH challenge experiment. Since defecation typically occurred in the kennels between blood sampling events, the exact time of deposition could not be recorded. Instead, if a fecal sample was detected when a bird was taken out for a blood sample, the time of deposition was recorded as the average of the time the bird had spent in the kennel since the previous blood sampling event. Fecal samples were transferred into plastic 12×75 mm test tubes (VWR International, West Chester, PA), and stored at −80 °C. The samples were dried using a Speed-Vac® Plus evaporator (SC110A; Savant Instruments, Holbrook, NY), crushed, and measured out in 0.02 to 0.03 g aliquots. Fecal samples were extracted as described by Monfort et al. (1998). Dried samples were reconstituted and extracted in 650 μL of methanol (MeOH), then 100 μL of extracted sample was transferred to a second set of test tubes that were dried using a manifold and reconstituted in 2 mL of buffer (ICN steroid diluent) yielding a 1:20 solution.

2.3. ACTH challenge

An ACTH challenge was performed to characterize the stress response in harlequin ducks. The first ACTH challenge study was performed in late April 2002. Birds in the treatment group (EXP, $n=7$) received an intra-muscular injection of 0.5 mL (25 mg) of synthetic adrenocorticotropic hormone (ACTH; Cortrosyn®, Organon Inc., West Orange, NJ, USA). Control birds (CTRL, $n=3$) received the same volume of sterile saline. Four of the ducks in the EXP group had to be removed from the study due to low (less than 30%) packed cell volume (PCV). When PCV levels had returned to normal (i.e. around 40%) three weeks later, these birds participated in a second ACTH challenge. Furthermore, two birds that had been in the treatment group were placed in the control group and two birds from the control group were injected with ACTH at the second ACTH challenge. Hence, four birds were subjected to both treatments for a total of nine birds in the EXP group and five in the CTRL group. Because the half-life of ACTH in ducks was measured at 10 min (Harvey et al., 1980), it was assumed that a second ACTH treatment or moving birds from the EXP group to the CTRL group had no effect on their response to the ACTH treatment. For "Time 0" samples, 0.2 to 0.7 mL of blood was drawn from each individual just prior to administration of ACTH or saline, and serial samples were drawn at 30, 60, 90, 120, 180, and 240 min after the injection. Fecal samples were collected during 0–8 h post ACTH, as described earlier.

2.4. Field samples

Serum samples were collected from wild harlequin ducks ($n=26$) captured in floating mist nets (Kaiser et al., 1995) in Prince William Sound, Alaska (60° N; 148° W) during late November and early December 2002. Birds were captured in nine locations in three general areas (Montague Island, Naked Island, and Green Island) of Prince William Sound. The mass, sex, capture location, and age class of each bird were recorded. Age classes were determined by inspection of bursal depth (Mather and Esler, 1999) and divided into hatch year (HY) and after hatch year (AHY) birds.

A blood sample was obtained within 3 to 18 min of capture and birds were placed in individual kennels. The time between capture and blood sampling was recorded for each bird. This was done to observe the relationship between time since capture and CORT concentrations. Only samples obtained within 3 min of capture were used to calculate the average baseline CORT concentration. Samples obtained within 3 min of capture are generally considered to represent baseline CORT concentrations in wild birds (Wingfield et al., 1982; Romero et al., 1997; Silverin and Wingfield, 1998; Romero and Romero 2002). Birds were implanted with abdominal VHF transmitters using previously described techniques (Korschgen et al., 1996). A second sample was obtained 3 to 6 h post capture to investigate the influence of a continuous stressor on CORT concentrations.

2.5. Radioimmunoassay

A double antibody radioimmunoassay (ImmuChem™ Double Antibody Corticosterone ^{125}I RIA Kit, ICN Biomedicals, Inc., Costa Mesa, CA) was used to quantify CORT concentrations in serum and GCM concentrations in fecal samples. The procedure provided by the manufacturer was followed except that all reagent volumes were halved and an additional standard (12.5 ng/mL) was produced by diluting the 25 ng/mL standard with the steroid diluent provided in the kit to increase the sensitivity of the assay. Standard validation procedures and quality controls were used including tests of parallelism and recovery of added corticosterone (O'Fegan, 2000). Parallelism was tested by comparing a 50 μL pool of harlequin duck serum or feces added to the kit standard calibrators with pure standard calibrators. The resulting standard curves were plotted after log-logit transformation (Rodbard, 1974). Serial dilutions (1:2 to 1:1024) of harlequin duck serum and fecal pools produced curves parallel to the standard curve. Mean recovery of added corticosterone was 101.3% (SD=8.5) for serum pools ($y=-2.19+1.022x$, $r^2=1.00$) and 80.6% (SD=14.44) for fecal sample pools ($y=-16.88+1.28x$, $r^2=0.99$). Serum samples were run at a dilution of 1:100 and fecal samples at a dilution of 1:20 in the assay buffer provided in the kit. Sensitivities for the serum and fecal assays were 15.4 ng/mL and 14.5 ng/g, respectively. Cross reactivity of the corticosterone antisera was reported by the manufacturer to be 100% with corticosterone and <1% for other steroids. Intra-assay and inter-assay variations were 2.9% and 9.0% for serum, and 3.0% and 6.1% for fecal samples, respectively.

2.6. High pressure liquid chromatography (HPLC)

In order to establish immunoreactive GC constituents in female harlequin duck serum and feces an HPLC (Varian

ProStar 210/215, Varian Inc., Walnut Creek, CA, USA) analysis was performed. Individual serum and fecal samples were selected at random and used to create 3 mL pools. The method used to analyze and collect fractions (1 to 80) of the serum and fecal pools by using HPLC has been previously described (Monfort et al., 1998; Mashburn and Atkinson, 2004). The immunoreactivity associated with CORT in the pools which co-elutes with radiolabeled (^{3}H) CORT on an 80 min HPLC gradient (flow rate 1 mL/min) was evaluated.

2.7. Statistical analyses

Statistical analyses were performed using SAS® and SigmaStat software. Concentrations from fecal samples were pooled into two-hour time blocks due to the uncertainty of the exact deposition time. A nonparametric analysis of variance (Kruskal–Wallis Test) was used to compare CORT concentrations among bleed times and treatments (serum samples). A regression analysis was used to investigate the relationship between time since capture and CORT concentrations for all field samples. A significance level of 0.05 was used for all statistical tests. All mean CORT concentrations are presented ± one standard error (SE).

3. Results

3.1. High pressure liquid chromatography (HPLC)

Pooled female harlequin duck serum exhibited a peak of immunoreactivity which co-eluted with tritiated CORT (Fig. 1). While similar elution profiles were observed between the fecal pool and tritiated CORT, a large immunoreactive peak in the polar regions (i.e., lower fractions numbers) of the elution gradient was also apparent (Fig. 1B).

3.2. ACTH challenge

Mean “Time 0” serum CORT concentrations did not differ between the experimental and control group (Fig. 2). Mean serum CORT concentrations for the controls did not vary from

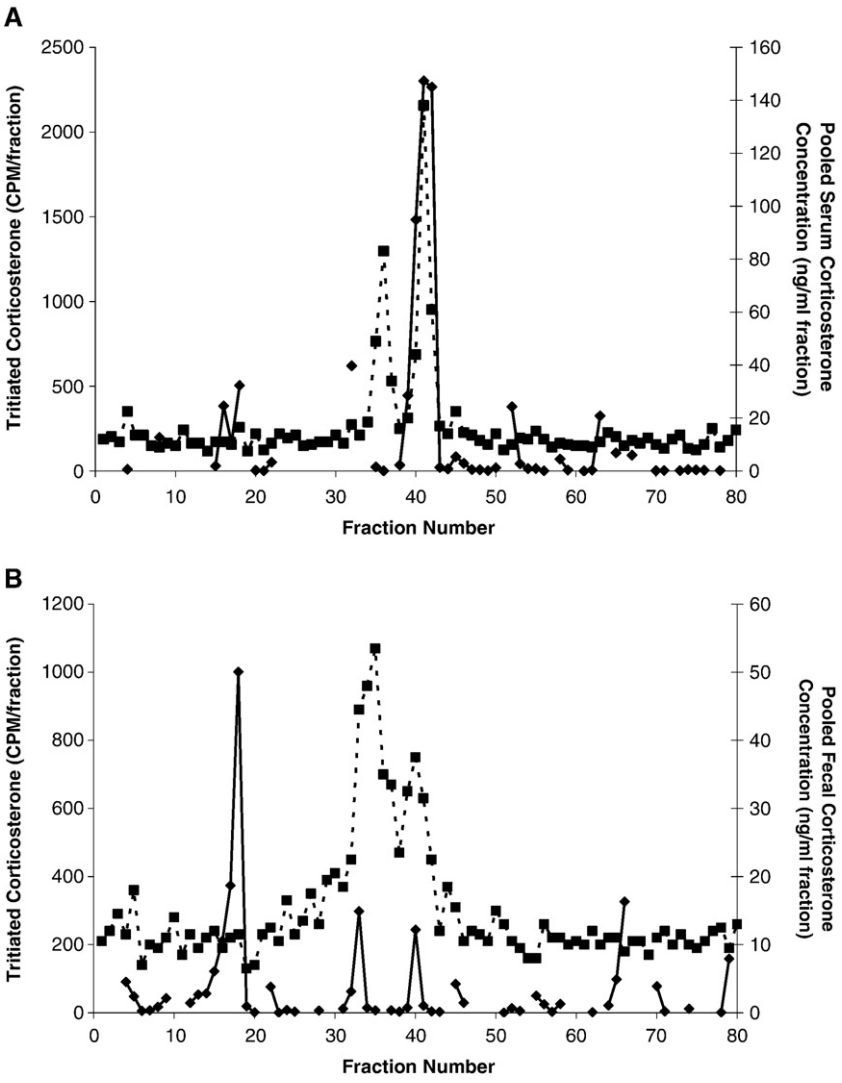

Fig. 1. Immunoreactive corticosterone HPLC profiles of harlequin duck (A) serum and (B) feces (solid lines). ^{3}H corticosterone (dotted line) was added as a reference.

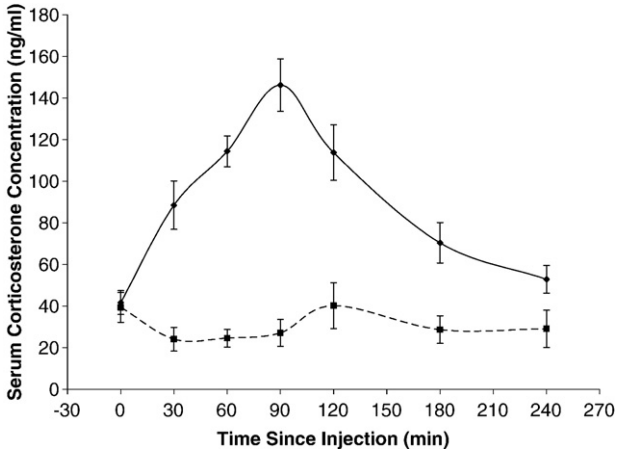

Fig. 2. Serum corticosterone concentrations (mean±SE) in harlequin ducks after injection of synthetic ACTH (solid line, $n=9$) or saline (dashed line, $n=5$). ACTH/saline injection at 0:00, first sample collected prior to injection.

"Time 0" concentrations (24.1 to 41.0 ng/mL) throughout the experiment (Fig. 2). The mean peak serum CORT concentration occurred 90 min post ACTH administration (Fig. 2). Mean peak CORT concentration was approximately three times higher than baseline (146±12.7 ng/mL). A significant difference between treatment and control groups was observed at 30 min ($P=0.0022$), 60 min ($P=0.0037$), 90 min ($P=0.0044$), 120 min ($P=0.0032$), and 180 min ($P=0.0136$) post ACTH. The final measurement (i.e., 240 min post ACTH) did not differ significantly from baseline.

Two to 13 fecal samples were obtained during each 2 h time block. Large individual variations in GCM concentrations were observed (Fig. 3). Mean fecal GCM concentrations in the ACTH group showed a four- to five-fold increase 2 to 4 h post injection (Fig. 3).

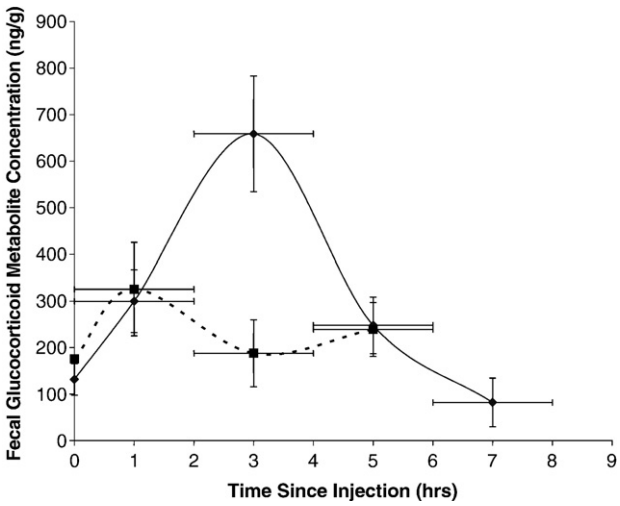

Fig. 3. Fecal glucocorticoid metabolite concentrations (mean±SE) in captive female harlequin ducks after injection of synthetic ACTH (solid line) and saline (dashed line). Fecal samples were pooled into two-hour time blocks due to the uncertainty of exact time of deposition within that period (horizontal bars).

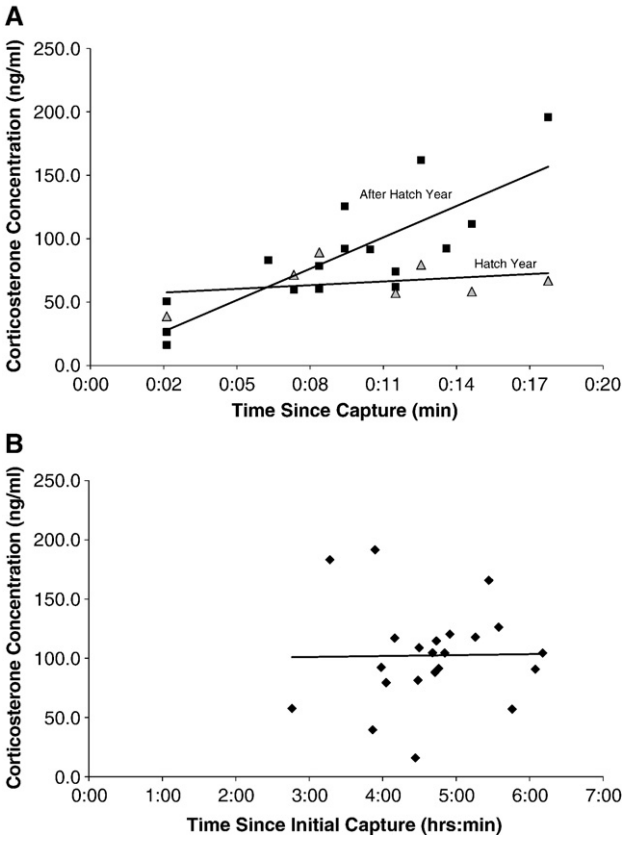

Fig. 4. Serum corticosterone response to (A) capture in hatch year (triangles; $n=7$; $y=1455.2x+54.681$, $r^2=0.093$, $P=0.0001$) and after hatch year birds (squares; $n=16$; $y=12365x+2.122$, $r^2=0.656$, $P=0.0001$) and to (B) short term captivity and surgery for all age classes (diamonds; $n=22$; $y=18.562x+98.825$, $r^2=0.0002$, $P=0.943$;), in wild harlequin ducks in Prince William Sound, Alaska (60° N; 148° W). Each data point represents a different individual.

3.3. Field samples

Serum CORT concentrations increased with time since capture (TSC) in samples obtained from AHY birds between 3 and 18 min of capture when CORT concentrations for each individual were plotted in a composite stress response curve (Fig. 4A). A positive relationship between time since capture and CORT concentration was found for AHY birds ($n=16$; $P=0.0001$; linear regression equation that best fit the data was CORT=2.122+8.587 TSC). The average CORT concentration in birds sampled within 3 min of capture was 18.7 (±10.2) ng/mL, however, sample size was low ($n=3$). Samples obtained between 3 and 6 h after capture exhibited high variability and no trend in CORT concentrations (Fig. 4B). However, 90.5% ($n=19$) of the samples showed elevated CORT concentrations with concentrations ranging between three and ten times the calculated baseline concentration.

4. Discussion

The co-elution of tritiated CORT and the peak of GC activity observed in both serum and fecal samples along with the tests for linearity and parallelism validated the RIA procedure

described as a method for measuring concentrations of GC in harlequin ducks. Along with routine quality control measures of non-specific binding, assay sensitivity and intra- and inter-assay variation, the assay can reliably describe adrenal activity in fecal and serum samples. Furthermore, through the validation procedure CORT was confirmed as the main GCM in harlequin duck serum, whereas a large polar peak in the elution gradient existed in the harlequin duck feces. In a similar study conducted on a marine mammal species, a polar peak in the elution gradient for cortisol and corticosterone also existed (Mashburn and Atkinson, 2004). That peak was subsequently identified as a corticosterone metabolite, likely a corticosterone glucuronide or corticosterone sulfate. While the polar peak in the harlequin duck feces may be a conjugated GCM, it was not confirmed in this study and it is possible that some of the fecal signal is not related to corticosterone release.

Whereas several studies have validated fecal GC assays for a variety of avian species: e.g. Florida sandhill cranes (*Grus canadensis pratensis*; Ludders et al., 2001), European stonechats (*Saxicola torquata rubicola*; Goymann et al., 2002), Mourning doves (*Zenaida macroura*; Washburn et al., 2003), Dickcissels (*Spiza americana*; Suedkamp Wells et al., 2003), California spotted owls (*Strix occidentalis occidentalis*; Washburn et al., 2004), to our knowledge validation of a fecal GC assay for a member of the sea duck tribe, *Mergini*, has not been published. The present study suggested that fecal samples could be used to non-invasively monitor adrenal activity in harlequin ducks.

The duration and the magnitude of the stress response following ACTH injection observed in the present study define the adrenal reaction to an acute stressor in harlequin ducks. These findings enable researchers to distinguish between ducks that are chronically stressed and birds that are responding to an acute stressor by defining the acute stress response curve. The observed three-fold increase of basal CORT concentrations following an exogenous ACTH injection is similar to results from American black ducks (*Anas rubripes*) (Spelman et al., 1995). Other avian ACTH studies have found a five-fold increase in Florida sandhill cranes (Ludders et al., 1998) to a nine-fold increase in domestic ducks (*Anas platyrhynchos*) (Harvey et al., 1980). The time lag between the stressor (ACTH injection) and the peak concentration in the present study was similar to the lag-time found in American black ducks (90 versus 120 min), but differs from that found in some other avian species: 270 min for Moluccan cockatoos (*Cacatua moluccensis*) (Walsh et al., 1985) and 60 to 90 min for bald eagles (*Haliaeetus leucocephalus*) (Zenoble et al., 1985b). The sharp increase and the subsequent gradual tapering off of CORT concentration observed in this study contrasts with the post ACTH CORT profile for breeding male Gambell's white-crowned sparrow (*Zonotrichia leucophrys gambelii*) where a steep increase in CORT during the initial 10 min was followed by a 2 hour plateau at elevated concentrations (Astheimer et al., 1994).

The absence of increased serum CORT in the harlequin duck control group indicates that these birds were not stressed by handling or blood draws. The birds participating in this experiment were handled on a weekly basis for 6 months prior to this study and were likely habituated to handling. Freeman and Manning (1979) reported a decreased CORT response in chickens after being handled twice a day for one week. el-Halawani et al. (1973) observed habituation in the CORT response to other stressors in turkeys over several weeks.

Fecal GCM concentrations in female harlequin ducks peaked between 2 and 4 h after ACTH injection, similar to Florida sandhill cranes, which were reported to peak around 2 to 3 h after ACTH administration with concentrations slowly tapering off over the next few hours (Ludders et al., 2001). Goymann et al. (2002) found fecal GC concentrations in European stonechats to peak at around 1 h 20 min post ACTH whereas the fecal GC concentration in northern spotted owls (*Strix occidentalis caurina*) increased steeply about 2 h after ACTH administration, with the peak concentration at 12 h post injection (Wasser et al., 1997). The observed four- to five-fold increase in fecal GCM concentrations from baseline to peak in the birds in the present study is comparable to those observed in other avian ACTH studies (Wasser et al., 1997; Ludders et al., 2001; Goymann et al., 2002). Although the metabolites measured by the RIA did not completely co-elute with labeled corticosterone, they reflected a physiological response to ACTH administration. Our findings provide evidence that fecal monitoring could be utilized to characterize stress responses in harlequin ducks.

The relationship between serum CORT concentrations and time since introduction of a stressor (i.e., capture) observed in harlequin ducks in PWS 2002 is characteristic of the adrenal stress response and has been shown for many avian species (Wingfield et al., 1982; Astheimer et al., 1995; Pravosudov et al., 2001; Cockrem and Silverin, 2002). However, the magnitude of the stress response was much greater in the present study as compared to a study on the same species in Washington by Perfito et al. (2002). At 15 min post capture, birds captured in Prince William Sound had increased in blood CORT concentrations from around 20 ng/mL to 100 ng/mL whereas the corresponding concentrations in the Washington study for molting ducks were around 25 ng/mL to 40 ng/mL in one of the study years. The highest CORT concentrations reported by Perfito et al. (2002) for molting harlequin ducks were approximately 45 ng/mL at 30 min post capture, after which the concentrations decreased. In contrast, harlequin ducks in the present study, after surgery and spending between 3 and 6 h in kennels, exhibited CORT concentrations that ranged from 16 to 192 ng/mL, with most (80.9%) being between 50 and 150 ng/mL. Whereas the specific reasons for the much higher CORT concentrations observed in the present study are unknown, they may include differences in capture method (Perfito et al., 2002; Romero and Romero, 2002), geographical location (Silverin et al., 1997; Silverin and Wingfield, 1998), season (Romero et al., 1997; O'Reilly and Wingfield, 2003), molt status, and RIA methods. Indeed, Perfito et al. (2002) found significantly higher basal CORT concentrations in breeding harlequin ducks caught in box traps versus mist nets. The average basal CORT concentration in the present study (16 ng/mL) was similar to concentrations for breeding birds caught in box traps (circa: 20 ng/mL) in the Washington study but slightly lower than molting ducks caught with the same method (Perfito et al.,

2002). However, Perfito et al. (2002) utilized a different assay. As different RIAs are known to give different absolute concentrations, caution must be used in comparing absolute concentrations between the two studies.

The older birds (AHY) exhibited strong correlations between time since capture and CORT during the first bleed after capture whereas hatch year birds did not, suggesting that the stress response may not be fully developed in harlequin ducks at 6 months of age. Studies investigating the stress response in developing chicks of several avian species have found that the stress response increases in magnitude and becomes more pronounced as a chick ages (Sims and Holberton 2000; Love et al., 2003; Walker et al., 2005).

Individual differences in the shape and magnitude of the stress response curve may account for some of the high variability observed in samples obtained 3 to 6 h post capture. Several studies report large variation among individuals of other bird species in basal CORT concentrations (Cockrem and Silverin, 2002; Vleck et al., 2000) as well as in the magnitude of the stress response (Littin and Cockrem, 2001). Different susceptibility to the stressors the birds were exposed to in this study (i.e., capture, bleed, surgery, and confinement for several hours) may be responsible for some of the variation observed. Although no clear trend or direct relationship between time spent in kennels and CORT concentrations could be found, the elevated concentrations observed in 19 of 21 birds between 3 and 6 h after capture indicate an adrenal response to a stressor. Interestingly, the mean CORT concentration observed in wild birds after 3 to 6 h of temporary holding was very similar to the maximum CORT response seen during the ACTH challenge of captive birds. Based on the results from our ACTH study, CORT concentrations should return to baseline concentrations about 4 h after exposure to an acute stressor. Instead, we observed elevated CORT concentrations, between three- and ten-fold over baseline, throughout the holding period (up to 6 h). The persistently elevated CORT concentrations observed in this study are likely a combination of the adrenal responses to capture, blood sampling, surgery, and confinement, however, the relative contribution of each is not known. Multiple stressors are known to interact in unpredictable ways (Dallman et al., 1992) and different stressors can elicit stress responses of varying magnitude (Sapolsky et al., 2000).

In conclusion, our results confirmed that CORT is the primary circulating GC in harlequin ducks, although a polar metabolite is likely the primary form in the feces. Commercially available RIA kits can be used to assess serum CORT and fecal GCM concentrations in harlequin ducks. In addition, fecal samples can be used as a non-invasive method to assess adrenal activity in this species. Injection of exogenous ACTH produced a three- and a four- to five-fold increase in serum CORT and fecal GCM concentrations, respectively. The relatively typical time (90 min) to maximum response to the ACTH injection and the duration of the response (up to 240 min) provide a definition by which the response to an acute stressor can be defined. Younger free-ranging birds did not exhibit a well defined adrenal reaction in response to capture whereas older birds did. Confinement in individual transport kennels and surgery resulted in elevated serum concentrations of CORT throughout the holding period. Since avian field studies employ a variety of techniques to capture and hold birds, studies addressing the CORT response to different methods of capture, post-capture confinement, and surgical procedures applied in the field would likely prove useful for interpretation of data and minimizing stress for birds.

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