Research article

Feather and faecal corticosterone concentrations predict future reproductive decisions in harlequin ducks (*Histrionicus histrionicus*)

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Understanding sources of reproductive variation can inform management and conservation decisions, population ecology and life-history theory. Annual reproductive variation can drive population growth rate and can be influenced by factors from across the annual cycle (known as carry-over effects). The majority of studies, however, focus solely on the role of current environmental events. Past events often influence future reproductive decisions and success but can be logistically difficult to collect and quantify, especially in migratory species. Recent work indicates that glucocorticoids may prove good indicators to evaluate carry-over effects across life-history transitions. Here, we evaluated three different measures of glucocorticoid physiology (feathers, faeces and plasma) to evaluate the predictability of future breeding decision in the harlequin duck (*Histrionicus histrionicus*). We collected tail and back feathers, plasma and faeces for glucocorticoid analysis, and fitted female harlequin ducks with very high-frequency transmitters to track their breeding decisions. Both back feathers (moulted immediately before the current season) and faecal glucocorticoid metabolites were identified as important predictive factors of reproductive decisions; high concentrations of glucocorticoid metabolites in back feathers and faeces predicted a higher likelihood of reproductive deferral for the year. Although back and tail feather corticosterone concentrations were correlated, tail feathers (moulted at the end of the previous breeding season) did not predict breeding decisions. Plasma corticosterone concentrations were collected over too broad a time range after capture to be useful in this study. This study demonstrates the utility of non-invasive corticosterone metrics in predicting breeding decisions and supports the use of feathers to measure carry-over effects in migratory birds. With this technique, we identified the prenuptial moult as an important life-history phase that contributes to reproductive decisions. Identification of critical life-history phases is paramount to efficient management of species.

Key words: Carry-over effects, glucocorticoid physiology, harlequin, reproduction, stress

Introduction

Understanding the causes of annual reproductive variation can inform management and conservation decisions, population ecology and life-history theory. Annual variation in predation, resource availability, weather or disturbance can drive variation in reproductive success (MacLulich, 1957; Coulson *et al.*, 2001; Visser *et al.*, 2004), while high variation in population demographics can lead to increased extinction risk in small populations (Boyce *et al.*, 2006). In particular, annual reproductive variation is a major contributor to population growth rate (Sæther and Bakke, 2000). Highly variable annual reproductive rates are typically displayed in longer-lived birds with high adult survival and late age of maturity (Erikstad *et al.*, 1998). In these systems, adult survival is selected for over reproduction, where many adults may breed in good years when resources are plentiful and defer reproduction in bad years when resources are poor (Stearns, 1992).

Studies exploring the basis for reproductive variability primarily evaluate current environmental events. However, carry-over effects are likely to be mediators of breeding success (Stearns, 1992; Webster *et al.*, 2002; Norris, 2005; Inger *et al.*, 2010; Harrison *et al.*, 2011). Carry-over effects are situations 'in which an individual's previous history and experience explains their current performance in a given situation' (working definition taken from O'Connor *et al.*, 2014). In seasonal organisms, conditions during the former breeding season, winter or migration can influence current reproductive success (see examples below); therefore, it is important to consider the role of carry-over effects in reproductive decisions. Unfortunately, it can be logistically difficult to determine resource and environmental conditions on the wintering grounds that may influence reproduction. Different physiological metrics, such as body condition and fat scores (Angelier *et al.*, 2011), have been used to infer environmental conditions outside of the breeding period, but these metrics can change rapidly and may not be informative if subjects are not captured immediately upon arrival. Other, more stable physiological records have been used to link large gaps in time to reproduction. For example, stable isotopes have been used to identify individuals in the breeding season that had access to high-quality forage during the previous winter (Marra *et al.*, 1998). Individuals with an isotopic signature of greater forage quality during winter arrived earlier at the breeding grounds (Marra *et al.*, 1998), probably increasing reproductive success (Lozano *et al.*, 1996).

Glucocorticoids have been widely used to quantify individual quality (condition and performance metrics) and predict fitness in individuals and populations (Marra and Holberton, 1998; Breuner *et al.*, 2008; Bonier *et al.*, 2009; Angelier *et al.*, 2010). Corticosterone (CORT) is the primary glucocorticoid released from the hypothalamic–pituitary–adrenal (HPA) axis in birds in response to perturbation. At baseline concentrations, CORT regulates diurnal activity and energy mobilization (Landys *et al.*, 2006). Activation of the HPA axis during stress increases CORT secretion to promote survival, but chronic secretion can have deleterious effects (Wingfield *et al.*, 1998). Studies of CORT have indicated that it plays an important role in mediating behaviour and reproductive decisions. For example, elevated CORT concentrations in laying barn swallows (*Hirundo rustica*) decreased offspring quality (Saino *et al.*, 2005). Experimentally elevated baseline CORT in female kittiwakes (*Rissa tridactyla*) during chick rearing initiated an earlier departure for the wintering grounds, and these females stayed on the wintering grounds longer than control birds (Schultner *et al.*, 2014). Several studies on seabirds have shown that individuals with higher baseline CORT during pre-egg lay were more likely to defer breeding (Goutte *et al.*, 2010a, b). These studies suggest that CORT mediates important life-history characteristics, but primarily examine effects within one life-history phase. The question remains whether CORT may be beneficial in evaluating carry-over effects across stages of the annual cycle.

The recent evaluation of CORT metabolite concentrations in feathers allows for the possible examination of carry-over effects; feather CORT metabolites are thought to represent an integrated metric of plasma CORT concentrations over the time the feather is grown through incorporation at the feather follicle during growth (Jenni-Eiermann *et al.*, 2015). High feather glucocorticoid concentrations from the autumn moult predicted lower winter survival in house sparrows (*Passer domesticus*; Koren *et al.*, 2012). A study of red-legged partridges (*Alectoris rufa*) revealed that higher CORT metabolites in feathers grown immediately after breeding reflects greater breeding effort (more young raised) from that year (Bortolotti *et al.*, 2008). In giant petrels (*Macronectes* spp.), greater CORT metabolites in feathers grown immediately after the breeding season reflect greater reproductive effort from that year, while also predicting lower reproductive success in the following year (Crossin *et al.*, 2013). Hence, feather CORT metabolites may represent conditions from a separate stage of the annual cycle, and therefore allow for incorporation of carry-over effects into determinants of current reproductive decisions.

Here, we evaluate whether three separate measures of CORT physiology (plasma CORT, faecal CORT metabolites and feather CORT metabolites) predict reproductive deferral in female harlequin ducks (*Histrionicus histrionicus*). The three metrics estimate CORT physiology over four separate time frames (i–iv below). Plasma CORT represents (i) the immediate state of the HPA axis, but may increase above baseline concentrations if animals are difficult to capture. Faecal CORT metabolites represent (ii) recent CORT secretion patterns [from the prior 2–4 h in harlequin ducks (Nilsson *et al.*, 2008)], independent of capture stress. Feather CORT metabolites represent an integration of CORT secreted over the time the feather was grown (Bortolotti *et al.*, 2008; Lattin *et al.*, 2011; Homberger *et al.*, 2015; Jenni-Eiermann *et al.*, 2015), and so can be used to evaluate the state of the HPA axis outside of the current season. Tail feathers are moulted at (iii) the end of the previous breeding season,

whereas back feathers are moulted (iv) immediately before migration to the breeding site. We predicted that greater CORT and CORT metabolites would correlate with reproductive deferral, representing either poor current conditions (with greater plasma CORT and/or faecal CORT metabolites) or poor conditions in other stages of the annual cycle having carry-over effects (with greater feather CORT metabolites).

Materials and methods

Study site and species

Data were collected in Glacier National Park (48°38′N, 113°52′W), Montana, during 2011–2013, on Upper McDonald Creek. This stream produces 25% of known harlequin duck broods in Montana and has the highest density of breeding harlequins in the lower 48 states (Montana Natural Heritage Program and Montana Fish, Wildlife and Parks, 2016).

Harlequin ducks (Fig. 1) are a migratory species with a Holarctic distribution. They winter along northern latitude coastlines and migrate inland to fast-moving streams to breed. Individuals are long lived (an 18-year-old male in the study population is one of the longest lived harlequin ducks on record) and form life-long pair bonds. Annual female survival averages 78%, based on data from two banded populations (Smith, 1998), and female breeding success is generally low until at least 5 years of age (Reichel and Genter, 1996). However, females can breed in their second year (Robertson and Goudie, 1999), and there are two accounts of yearling females breeding. Reproductive deferral is common in this long-lived species (Bengtson and Ulfstrand, 1971). Over 20 years of surveys on this population indicate that, on average, only ~30% of the paired females produce broods (range, 0–100%; W. K. Hansen, unpublished data). Studies on other populations indicate similar low brood production by paired

Figure 1: Breeding pair of harlequin ducks (*Histrionicus histrionicus*) on the breeding grounds on Upper McDonald Creek, Glacier National Park, MT, USA. Photograph courtesy of John Ashley.

females [16% of all females present hatched young in an Icelandic population (Gardarsson and Einarsson, 2008); 50% of marked females hatched young in a population in Quebec (Brodeur *et al.*, 2008)]. These numbers do not account for nest failure during incubation (females that did not defer egg lay but failed to bring young to hatch). However, in the present study, telemetry data indicate that only 25% of radio-tagged females attempted reproduction, with the other 75% deferring reproduction (W. K. Hansen, unpublished data).

Only female harlequins incubate and care for young (Bengtson, 1972; Rohwer and Anderson, 1988). Males return to the wintering grounds soon after females initiate incubation, allowing for only one reproductive attempt per year. Females are primarily income breeders; nutrients required for egg production are acquired from the breeding stream (Bond *et al.*, 2007). Females lay one to seven eggs in a ground nest close to the stream edge (Kuchel, 1977). Chicks emerge on the stream from mid-July to early August and remain on the stream until September, when their mothers escort them to the wintering grounds. Upon arrival at the wintering grounds (September–October), females undergo a pre-basic moult, in which all feathers are replaced. Immediately before migration to the next breeding season (end of March–April) females undergo the prenuptial moult and replace only body plumage (Fig. 2; Pyle, 2008).

Sample collection

The protocol was approved by the University of Montana Institutional Animal Care and Use Committee (AUP 011-11), the National Park Service and the US Fish and Wildlife Service. Pairs were captured on the breeding stream shortly after arrival (from April into May), prior to egg lay. Nest initiation in this region ranges from late May to early June, depending on snowpack and water runoff (Diamond and Finnigan, 1993; Smith, 1998). Ducks were captured using 3 m × 18 m mist nets (see Smith *et al.*, 2015). Upon capture, blood was collected from the alar vein using a 30 gauge needle and heparinized microhaematocrit tubes. Collection of a baseline CORT sample in <3 min is necessary to measure baseline CORT (Perfito *et al.*, 2002; Romero and Reed, 2005). However, given the difficulty of netting birds across fast-moving, high-water streams, the majority of blood samples were collected well after the 3 min window (Fig. 3). Blood samples were kept on ice until centrifugation later that same day; plasma was then removed and stored at −20°C. Birds were weighed to the nearest 5 g using a 1000 g Pesola spring scale, and bill and tarsus measurements were made to the

Prebasic moult	WINTER	Prenuptial moult	BREEDING
S O N	D J F	M	A M J J A

Figure 2: Moult chronology of female harlequin ducks. Month abbreviated with the first letter, starting in September and ending in August. Timing of moult taken from Pyle (2008).
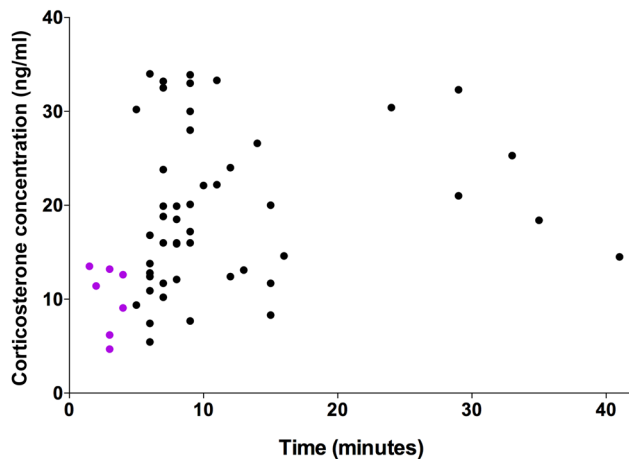
Figure 3: Distribution of plasma corticosterone concentrations since time of capture (in minutes). $n = 57$; purple circles indicate bleed times <4 min.

nearest 0.1 mm using dial callipers. Each bird received a US Fish and Wildlife Service band and a plastic blue and white alpha–alpha leg band. Two back feathers and one outermost right tail feather were collected and stored at –20°C until analysis. Faecal samples were collected opportunistically during handling and frozen the same day at –20°C until analysis. Females received an 8.5 g ATS very high-frequency (VHF) transmitter so that they could be tracked throughout the breeding season. When a female was found incubating eggs, she was classified as having made the decision to reproduce. Non-breeding females were tracked two or three times per week throughout the spring and summer to ensure that they were not incubating and were never found with chicks later in the season. Four females were sampled in all 3 years of the study, eight females were sampled in 2 of the 3 years, and 29 females were sampled during only 1 year of the study.

Hormone assays

We measured plasma CORT concentrations using enzyme-linked immunoassay (Enzo Life Science catalogue no. 901-097). We optimized the assay for harlequin ducks following Breuner *et al*. (2006). We assayed plasma at a final dilution of 1:40 with 1% steroid displacement buffer.

Feather corticosterone extraction

We measured feather CORT at the Centre d'Études Biologiques de Chizé, France and in the Breuner Laboratory in Missoula, MT, USA. We used radioimmunoassay following Bortolotti *et al*. (2008), but modified this method for whole feather extraction, as preliminary tests comparing cut with whole feathers showed no difference in extraction efficiency (whole feathers, 0.60 ± 0.09 pg/mm and cut feathers, 0.51 ± 0.06 pg/mm; Mann–Whitney $P = 0.49$; in future research this should be tested for each species, as larger feathers may not give the same result). Briefly, we removed the

calamus and measured the feathers (two back feathers and one tail feather for each individual) to the nearest 1 mm and 0.1 mg. Weight and length variation among samples was small [back feathers, $n = 82$, 5.2 ± 0.27 mg, 41.6 ± 1.14 mm (mean ± SEM); and tail feathers, $n = 102$, 29.7 ± 1.5 mg, 50.0 ± 1.3 mm]. We extracted CORT from whole feathers with 5 ml of 99.99% pure methanol in a plastic-capped glass test tube overnight in a 50°C water bath; the level of methanol covered the entire feather. Methanol was poured directly off the feather, and then evaporated off in a 50°C water bath under nitrogen. Extract residues were reconstituted in 300 µl phosphate-buffered saline with gelatin. If samples were not assayed the same day, they were frozen at –20° until analysis. Hormone recoveries were measured in all feather samples by adding a spike of ^{3}H-CORT (2000 c.p.m./50 µl) in the initial methanol incubation. Extraction efficiencies ranged from 100 to 58%, with a mean of $94 \pm 10.8\%$. We report feather CORT in picograms per millimetre of feather, as recommended by Lattin *et al*. (2011) and Berk *et al*. (2016).

Faecal corticosterone extraction

We measured faecal CORT following Hayward *et al*. (2010), with alterations based on personal communication from K. Hunt (New England Aquarium, Boston, MA). Briefly, we dried samples at 35°C overnight or until sample mass remained constant. Dried samples were sieved through a small-gauge screen to remove gravel and other particulate matter. Dried and sieved samples were weighed and incubated in ×20 volume of 99.99% pure methanol overnight in a 50°C water bath. Samples were then sent through a vacuum filtration system that removed particulate matter. Methanol was evaporated off the filtered samples in a 50°C water bath under nitrogen. Extracted residues were reconstituted in 300 µl phosphate-buffered saline with gelatin.

Radioimmunoassay

Feather and faecal CORT extracts were measured using radioimmunoassay with a highly cross-reactive antibody from Sigma (C8784), appropriate when measuring corticosterone and its metabolites (Lattin *et al*, 2011). Briefly, samples were incubated with 100 µl 1:100 antibody dilution and 100 µl 4000 c.p.m. ^{3}H-CORT. The standard curve ranged from 7.5 to 2000 pg/100 µl; external standards (for inter-assay variation) and blanks were run with each assay. Inter- and intra-assay coefficients of variation for feather assays were 2.8 and 19.8%, respectively. Inter- and intra-assay coefficients of variation for faecal assays were 2.1 and 0.47%, respectively.

Data analysis

All statistical analyses were performed with R 3.0.2 (R Core Team, 2013) and GraphPad Prism (v6.01). Blood samples ($n = 57$) were collected between 1 and 41 min after capture (Fig. 1); we fitted Cort by minutes after capture (with bird identity and year as random factors) with a quadratic function to evaluate whether correcting for time of capture could produce

Table 1: Harlequin duck (*Histrionicus histrionicus*) Akaike Information Criterion table predicting reproductive decisions (yes/no)

Fixed effects	Random	Model no.	No. of parameters	AICc	ΔAICc	wAICc
fback + FGM	ID + YR	1	5	30.29	0	0.68
fback + BCI	ID + YR	2	5	32.36	2.07	0.24
FGM	ID + YR	3	4	35.82	5.53	0.04
fback + FGM + BCI	ID + YR	4	6	36.28	5.99	0.03
ftail + FGM + BCI	ID + YR	5	6	42.39	12.1	0.00
Null model	ID + YR	6	3	66.0	35.71	0.00

Abbreviations: BCI, body condition index; fback, back feathers; FGM, faecal glucocorticoid metabolites; ftail, tail feathers; ID, bird identity; wAICc, model weight; YR, year.

meaningful residuals from the mean CORT concentration at that time. However, minutes since capture only explained 14% of the variation in plasma CORT ($n = 55$, $r^2 = 0.14$ for a quadratic fit), so the data were not analysed further.

With several measures of CORT physiology from each individual, we can evaluate whether these measures are repeatable within an individual across CORT measures (faecal vs. tail feathers vs. back feathers). We calculated the intraclass correlation coefficient across all three metrics; variables were scaled prior to analysis. We also estimated correlations between variables to avoid collinearity of variables in the AIC analysis.

Predictors of reproductive decision (dependent variable) included back feather CORT (in picograms per millimetre; $n = 52$), tail feather CORT (in picograms per millimetre; $n = 54$), faecal CORT (in nanograms per gram; $n = 32$) and body condition index (scaled mass index according to Peig and Greene, 2010, using a principal components analysis of tarsus and head/bill length for structural size, $n = 48$). We also included the year of capture and individual identity as random effects. We used a general linear mixed model (glmm) from the lme4 package in R, and selected the top models using an Akaike Information Criterion (AICc) approach (using AICcmodavg in R). All interactions were tested on the same data set; only a subset of models (representing the most interesting comparisons) are shown in Table 1. Age could play a significant role in helping to explain variation in our data, because glucocorticoid physiology is known to vary with age (e.g. Heidinger *et al.*, 2006). However, this population was unbanded prior to our first season (excluding a few individuals banded on their wintering grounds in Washington), and so we do not have ages to use in the analysis.

Results

We sampled 41 unique adult female harlequin ducks during spring trapping events from 2011 to 2013 (12 females were resampled across years, resulting in 57 sampling events). During this period, 10 unique females built nests and then laid and incubated eggs (resulting in 14 nests over the 3 year study period).

## Correlations between corticosterone metrics within individuals

The intraclass correlation coefficient (0.52) did not provide strong support for co-variation of CORT measures (back feather, tail feather and faecal) within individuals.

Breeding decision

The decision to breed was best explained (lowest AICc) by back feather CORT and faecal CORT [faecal glucocorticoid metabolites (FGM)], including year and bird identity as random factors (Table 1); this model improved significantly on the null model and was more than two points lower AICc than the next strongest model (Burnham and Anderson, 2002), and model weight (~70%) suggests that it is the best model of the set of candidate models (Burnham and Anderson, 2002). Tail and back feather CORT metabolites were collinear (lm $F = 7.38$, $P > 0.01$), and so were not included together in the AICc analysis; replacement of tail feather with back feather CORT data improved AICc by more than six points. Females with confirmed nest sites had lower concentrations of both back feather CORT metabolites and FGM (Fig. 4), indicating that both recent conditions (FGM) and carry-over effects from the prenuptial moult (back feathers) influence breeding decisions. Mean FGM concentrations do not appear different when viewed as means including all 3 years, but when plotted by year, we see that higher FGM concentrations predict reproductive deferral within year (Fig. 4).

Discussion

Increased back feather CORT metabolites and faecal glucocorticoid metabolites predicted subsequent reproductive deferral in female harlequin ducks. Back feathers are grown during the prenuptial moult completed on the wintering grounds immediately before the spring migration and are thought to represent an integration of plasma CORT present
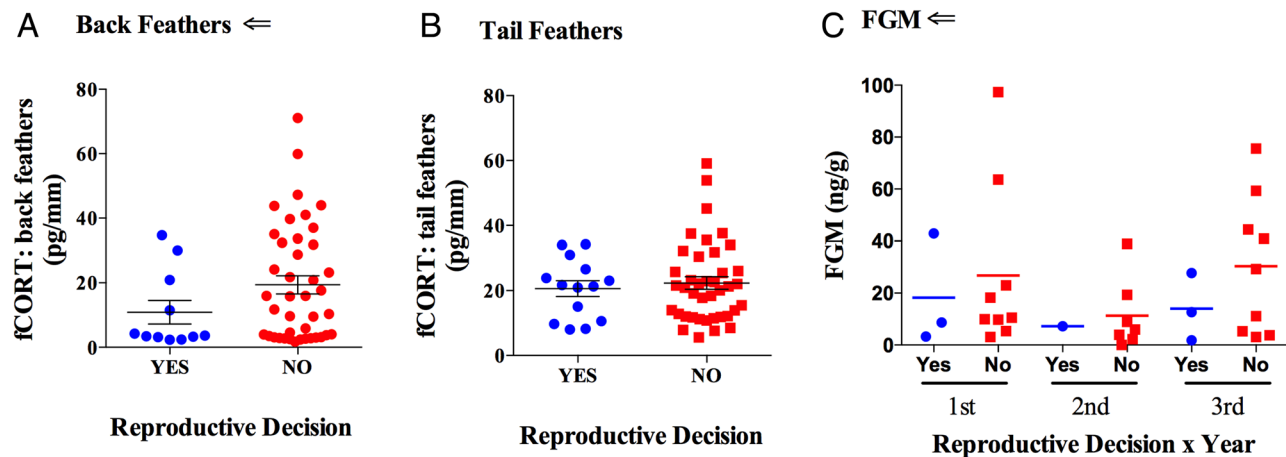
Figure 4: Distributions of female corticosterone metabolite concentrations and decision to reproduce (blue = yes, red = no) for back feathers (**A**; $n = 11, 41$), tail feathers (**B**; $n = 14, 40$) and faeces (**C**; $n = 7, 25$). Mean ± SEM shown in black. Arrows identify factors in the top model.

over the period of feather growth (Jenni-Eiermann *et al.*, 2015). Faecal glucocorticoid metabolites are thought to represent an integration of circulating plasma CORT concentrations over the previous 2–4 h in harlequin ducks (Nilsson *et al.*, 2008). Therefore, our data support both current environmental events and carry-over effects from the pre-migratory period as important in determining reproductive decisions in harlequin ducks.

Feather corticosterone and carry-over effects

Carry-over effects are seasonal interactions where phenomena during one season alter performance in another season (as per Norris, 2005; Harrison *et al.*, 2011; O'Connor *et al.*, 2014). Harlequin ducks moult their body feathers in the 2 weeks prior to inland migration to breed (Pyle, 2008). We found that females experiencing increased corticosterone during prenuptial moult (and therefore having elevated CORT metabolites in their feathers) are less likely to breed in the subsequent season. This indicates that a challenge in the local moulting environment carries over to influence reproductive decisions that year.

We did not determine the cause of increased feather CORT metabolites in deferring females. Sources of stress during the prenuptial moult could include adverse weather conditions, poor forage quality, con- and/or heterospecific social interactions, predator pressure or human disturbances (reviewed by Wingfield *et al.*, 1997). The relationship between high feather CORT and deferred reproduction is also likely to be very complex. Periods of extreme stress or chronic stress can have many detrimental effects on reproduction (Wingfield and Sapolsky, 2003). Although Wingfield and Sapolsky (2003) point out that there are evolved mechanisms to overcome stress, long-lived animals, such as harlequin ducks, may simply choose not to reproduce when conditions are not ideal. This observation has been made in many species of long-lived

sea birds (reviewed by Erikstad *et al.*, 1998; Goutte *et al.*, 2010a, b).

We found no relationship between tail feather CORT and reproductive deferral, indicating no carry-over effects between the prebasic moult and future reproduction. It is interesting that body feather CORT predicts reproductive deferral, whereas tail feathers do not. Both feathers are grown on the wintering grounds, but separated in time. Although CORT metabolite concentration is correlated across the two feather types, only back feather CORT predicts reproductive deferral. It is possible that food availability changes with season (autumn vs. spring) on the wintering grounds, altering energetic state and therefore glucocorticoid physiology across the two moults. However, it is also possible that the pre-basic (post-reproduction and autumn migration) moult is more heavily influenced by the previous reproductive period. In several studies, feathers grown in the pre-basic moult reflect reproductive effort from that season (Bortolotti *et al.*, 2008; Crossin *et al.*, 2013). By the time the prenuptial moult occurs, there is likely to be less influence of the previous reproductive period on energetic state and glucocorticoid physiology.

Faecal glucocorticoid metabolites

Higher FGM concentrations predicted reproductive deferral, indicating that current environmental events influence reproductive decision. Studies evaluating factors within one season (non-carryover studies) often find links between corticosterone physiology and reproductive decisions. For example, CORT implants decrease parental care and increase nest abandonment in pied flycatchers (Silverin, 1986), and endogenous free CORT concentrations are elevated in female European starlings that abandon their nests in the next 24 h (Love *et al.*, 2004). There are many examples of elevated (stress-induced) glucocorticoids inhibiting some aspect of reproductive function (reviewed by Wingfield and Sapolsky,

2003; Breuner, 2010). Faecal glucocorticoid metabolites and plasma CORT both evaluate the current state of the HPA axis; here, we measured FGM to evaluate current HPA axis function in harlequins, representing a more integrated view of CORT concentrations over the previous hours than is possible to obtain with plasma CORT. Our data do not support a direct connection between increased glucocorticoids and the decision to defer reproduction; they only suggest that environmental and/or physiological factors that may influence the decision to reproduce have also increased glucocorticoids.

Conservation management implications

In this study, we evaluated the possibility of four different glucocorticoid measures serving to identify the likelihood of reproductive deferral in harlequin ducks, incorporating both current measures (plasma CORT and FGMs) and historical measures (back and tail feather CORT metabolites). Our study has important implications for how and when we can use CORT physiology as a biomarker of reproductive success. First, our data point to a combination of current and carry-over effects influencing reproductive deferral in this population. Conservation management strategies typically mediate/restore conditions at the breeding grounds for species at risk. Inclusion of carry-over effects, however, opens up new habitats for consideration in species management. In the present study, higher CORT metabolites in back feathers indicate that conditions at the wintering grounds during the prenuptial moult might be suboptimal for female harlequins, and mediation of that habitat might be beneficial for reproductive output.

Second, our data suggest that the utility of plasma CORT concentrations is low in species that are difficult to capture. Plasma samples are often difficult to obtain from species at risk, and it is therefore tempting to use them if we have them at all. However, if the data cannot be corrected for time of capture to provide meaningful residuals, then it is misguided to incorporate them into management strategy recommendations. In Glacier National Park we net harlequin ducks on Upper MacDonald Creek, which is often >18 m wide and too deep and fast moving to stand in early in the season. Hence, the time from net capture to blood sampling was usually more than the 3–4 min window required to obtain baseline plasma CORT concentrations (Romero and Reed, 2005). Given that our data did not contain a strong signal of minutes after capture, we could not use these samples in our analysis.

Harlequin duck populations have been identified as 'threatened', 'at risk', 'sensitive' and 'of special concern', depending on the country and location of the population (Montana Natural Heritage Program and Montana Fish, Wildlife and Parks, 2016; Goudie, 2014). This species is long lived, slow to mature, requires a pristine breeding environment and reproduces on a boom-and-bust cycle, with a sporadic high-productivity year separated by years of low productivity (Montana Natural Heritage Program and Montana Fish, Wildlife and Parks, 2016). As a result, several local breeding populations have been extirpated throughout Colorado, Idaho and Montana (Robertson and Goudie, 1999). Recent work indicates that climate change may reduce overall reproductive success in harlequin ducks (W. K. Hansen, unpublished data), increasing the need for management strategies to maintain critical populations. Our data indicate that both current and previous environmental conditions influence reproductive decisions in the Montana population. It would be interesting to evaluate CORT physiology over boom and bust years to determine whether differences in CORT metrics across years can predict boom or bust reproductive output. However, although we have large variation in reproductive output since 1990 in our population (the percentage of pairs producing broods varies from 0 to 100%; W. K. Hansen, unpublished data), in the 3 years when we collected CORT data, the entire population averaged ~30% of pairs producing broods each year. Hence, we do not have boom and bust years represented in this data set.

Conclusion

This study demonstrates the utility of feather CORT and faecal glucocorticoid metabolites for predicting reproductive decisions in a migratory species. With these techniques, we suggest that both current and prior environments contribute to reproductive decisions in harlequin ducks. Identification of critical stages in the annual cycle is paramount to efficient management of species (Bump *et al.*, 1947; Hooper *et al.*, 1979; Heppell, 1998; Garrabou and Harmelin, 2002; Winemiller, 2005). We have shown a difference in the mean concentration of feather CORT and FGM between females that nested and females that did not nest, but there is a high degree of overlap in CORT concentration between these two categories of females. This suggests that CORT concentrations are not the only driver of reproductive variation within this population. Many other factors are likely to be influencing reproductive decisions. Additionally, we note that this study is entirely correlational. In conservation studies, we are often limited in experimental manipulations on species of concern. We were unable to manipulate CORT concentrations in this population, given its location in a National Park. Overall, feather and faecal CORT metabolites have provided insight into harlequin duck breeding biology, and suggest directions for management if numbers decline.

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