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Method Article

METHODS: Validating an immunoassay to measure fecal glucocorticoid metabolites in yellow-bellied marmots

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ABSTRACT

Quantifying physiological stress in wild animals is essential for understanding their health, reproductive success, and survival in a variable environment. The yellow-bellied marmot (*Marmota flaviventer*) study at the Rocky Mountain Biological Laboratory near Crested Butte, Colorado, USA is the world's second longest study of free-living mammals. Historically, we used a validated corticosterone radioimmunoassay (RIA) to measure fecal glucocorticoid metabolites (FGMs) as a proxy for physiological stress. However, the costs and risks associated with working with radioisotopes drove us to consider a more sustainable method. Here we evaluate the suitability of two competitive corticosterone enzyme assays (EIA), one from Cayman Chemical Company (CCC) and one from Arbor Assays (AA), to measure marmot FGMs via their cross-reaction. The findings revealed that the AA EIA better matched the RIA in terms of accuracy across high and low FGM concentrations, had superior assay parameters, showed the highest correlations with RIA results and effectively captured the annual variations in FGM concentrations, thus demonstrating its reliability for use in longitudinal studies. We further analytically validated the AA EIA for FGMs and confirmed its efficacy and lack of matrix effects, thus establishing its suitability for ongoing and future studies of FGMs in marmots. The transition to the AA EIA from the RIA ensures continued data integrity while enhancing safety and environmental sustainability.

1. Introduction

The precise measurement of physiological stress levels in wild animals is critical, not only for understanding individual and population health but also for gaining insights into broader ecological dynamics (Dantzer et al., 2016; Kroeger et al., 2021; Pinho et al., 2019; Price et al., 2018). Monitoring stress levels can help us understand how environmental pressures such as habitat degradation, pollution, or predation impact a population's viability (Sheriff et al., 2011). Furthermore, physiological stress can serve as a key indicator of ecological disturbance, allowing us to assess the stability and functionality of ecosystems (Boonstra, 2012; Karaer et al., 2023; Romero and Wingfield, 2015; Sapolsky et al., 2000; Sheriff et al., 2011). Therefore, a comprehensive understanding of physiological stress in wild animals can better inform conservation efforts and ecosystem management strategies, ultimately contributing to the preservation of biodiversity. Such data becomes even more useful when it is collected over long periods of time to assess trends.

Radioimmunoassay (RIA) and enzyme immunoassay (EIA) are both immunoassays used for measuring a biomarker of interest, such as stress hormones. RIA, one of the first immunoassay techniques developed, uses radioactively labeled antigens to detect the presence of antibodies in a sample. The degree of radioactivity allows for quantification of the antibody concentration (Goldsmith, 1975). By contrast, an EIA uses an enzyme-linked antigen or antibody as a marker instead of a radioactive isotope. The enzyme, in the presence of its substrate, produces a measurable product, typically a colour change, that correlates with the substance concentration in the sample (Lequin, 2005). Both methods

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rely on the specific binding between the antigen and the antibody but differ in their detection strategies—RIA using radioactivity and EIA using a colorimetric change.

Traditionally, RIAs have been used extensively due to their early development and initial sensitivity for measuring corticosterone and other glucocorticoid metabolites (Hare et al., 2014; Keay et al., 2006; von der Ohe et al., 2004; Wasser et al., 2000). However, RIAs are no longer as widely available, present greater safety and sustainability concerns, and necessitate strict radioactive handling regimens which renders them less convenient than EIAs. On the other hand, EIAs have become more popular due to their non-radioactive nature, making them safer and easier to handle. Furthermore, EIAs can be performed in different formats such as direct, indirect, sandwich, or competitive, each suited for different biomarkers (Lequin, 2005).

For the past 20 years the Blumstein lab has been studying stress in yellow-bellied marmots (*Marmota flaviventer*) using a corticosterone radioimmunoassay (RIA; MP Biomedicals) via its cross-reaction with fecal glucocorticoid metabolites (FGMs) extracted from fecal samples following (Smith et al., 2012). This study population, located around the Rocky Mountain Biological Laboratory near Crested Butte, Colorado, USA is the world's second longest, continually studied mammal (Blumstein et al., 2013). Although we do not know the specific molecular composition of marmot FGMs, quantification of stress using a corticosterone RIA was biologically validated using ACTH challenges and live-trapping in yellow-bellied marmots—results confirmed that the FGMs tend to cross-react well with antibodies raised against corticosterone and the peaks in detection matched stressed marmots (Smith et al., 2012). This suggests that although the RIA kit is designed to specifically measure corticosterone, it can still accurately depict stress levels through the antibody's cross-reaction with FGMs. Additionally, we acknowledge that we have not ascertained which specific FGMs the RIA cross-reacts with.

When the lab had to halt analyses using RIAs due to unforeseen circumstances, we decided to turn to EIAs. However, given the longitudinal nature of our data, it was important that this new data source was comparable to the previous analyses to maintain data continuity. Thus, to continue harnessing two decades of results while switching to safer, more convenient and sustainable methods, and to guarantee data accuracy, we compared the performance of two corticosterone EIA kits—one from Cayman Chemical Company (CCC) and one from Arbor Assays (AA)—to that of our old RIAs, using a mix of samples from 2016 to 2020. The goal was to calculate a correction factor that allowed us to use our previously collected samples in future studies. More specifically, we conducted a detailed comparison of FGM concentrations, a thorough examination of assay parameters, and a robust correlation and covariate analysis. We used these analyses to select the EIA kit best suited for us and followed this up with a rigorous analytical validation (spike recovery, dilutional linearity, and parallelism) to ensure the accuracy and reliability of long-term stress data in yellow-bellied marmots.

While several studies have made a similar switch from RIA to EIA (Al-Dujaili et al., 2009; Elder et al., 1987; Glucs et al., 2018; Kinn Rod et al., 2017), they do not usually involve such a long-term data set. Furthermore, many EIAs are validated and used for blood plasma, while there is less research that has been published on the use of fecal samples. FGMs are particularly useful proxies of glucocorticoids (GCs; e.g., corticosterone, cortisol) because feces are easy to obtain through minimally invasive and non-invasive sampling in wild animal population. Lastly, no research has been published that validates EIAs for measuring FGMs from yellow-bellied marmots.

2. Materials and methods

2.1. Sample collection and FGM extraction

We collected fecal samples from wild yellow-bellied marmots in the vicinity of the Rocky Mountain Biological Laboratory (RMBL), located in the East River Valley, in Gunnison County, Colorado USA. Fecal samples have been collected from this population since 2002. During the core active season (May to August), marmots are captured on a bi-weekly basis using Tomahawk traps positioned at burrow entrances. Upon reaching each trap, the marmots were carefully transferred into canvas handling bags for weight measurement and sex determination. At the time of initial capture, all marmots were uniquely marked using Nyanzol cattle dye and uniquely numbered ear tags (Armitage, 1982). Using a plastic Ziplock bag, fecal samples were collected from the trap (typically within 2 h post-defecation) or, opportunistically, directly from the bagged marmot and immediately stored on wet ice until they were transported back to the lab and frozen at −20 °C. Annually, in August, the samples are transported on dry ice to the Blumstein Lab at the University of California, Los Angeles (UCLA) for hormone extraction (as described in (Smith et al., 2012). Hormone extracts are then stored in ethanol at −20 °C.

2.2. Corticosterone assessment

From the fecal extracts, we measured FGMs via their cross-reaction with a corticosterone RIA from MP Biomedicals (MPB; Cat # 0712010-CF) and two different competitive corticosterone EIAs, one from CCC (Cat # 501320) and one from AA (Cat # K014-H1/H5). The RIA samples were diluted 4-fold, whereas CCC and AA samples were diluted 8-fold. All further immunoassay steps were conducted according to the manufacturer's instructions. Data from the CCC immunoassay was analyzed according to the manufacturer's protocol. Data from the AA immunoassay was analyzed using the My Assays LTD website provided by the manufacturer (MyAssays Ltd): https://myassays.com/arbor-assays-corticosterone-enzyme-immunoassay-kit-high-sensitivity.assay.

In total, we ran 144 distinct samples (from 92 unique individuals), across five different years (2016–2020) for the RIA. Three of these samples exceeded the limits of the AA, and 50 of them (including two full plates) exceeded the limits of the CCC assay. Given that two of the CCC plates failed to provide usable results, we then decided to run a final plate (24 samples) using only the AA assay (totaling 144 samples) to confirm its positive performance. The total number of samples that were successful on all three assays was 70. These samples included 40 females and 30 males; and 36 adults, 12 juveniles, and 16 pups (the rest were of unknown age, but likely adults).

2.3. Assay parameters

For the two EIAs, we calculated the limit of blank (LoB), lower limit of detection (LLoD), lower limit of quantification (LLoQ) and upper limit of quantification (ULoQ) as described previously (Armbruster and Pry, 2008; Taha, 2024) and the intra- and inter-assay coefficient of variations (CV) using the standard curve from four different runs. Total (TE) and relative (RE) error were calculated as described previously (Dutta et al., 2023; Kat and Els, 2012; Taha, 2024; Westgard et al., 1974).

2.4. Statistical analysis

We performed Pearson's correlation tests to assess the associations among FGM concentrations obtained from the three immunoassays. These models were also employed to determine the coefficient of determination (R^2), which assesses the proportion of variance explained in the FGM concentrations, and to evaluate the parallelism (see below) between the standard calibrators and the diluted samples using the AA immunoassay. Separately, we also used the *lme4* package (version 1.1–35.1; Bates et al., 2015) to fit mixed effects models to determine whether the FGM concentration of each assay was being influenced by sex or age. In these models we also included year and ID as random factors. All analyses were conducted in RStudio version 4.3.1 (Team, 2023).

2.5. Spike recovery

To test spike recovery on our selected AA EIA, fecal lysates were diluted ≤ the hypothetical lower limit of quantification (LLoQ) using the assay's buffer to avoid exceeding the ULoQ signal (Smith et al., 2012), and each sample was spiked with 39.0 pg/mL (low spike), 157.0 pg/mL (medium-low spike), 595.0 (medium-high spike), and 1337.0 pg/mL (high spike) of the recombinant standard calibrator provided with the EIA from AA. Percentage recovery was calculated using the below formula.

$$\frac{\text{Crude sample diluted to LLOQ} + \text{spiked sample}}{\text{Theoretical concentration}} \text{X } 100$$

2.6. Dilution linearity

To test dilution linearity on our selected AA EIA, undiluted fecal lysates were spiked to the ULoQ using the highest recombinant standard calibrator and diluted 2-, 4-, 8- and 16-folds in the assay's buffer. Percentage recovery was calculated using the below formula.

$$\frac{\text{Diluted sample}}{\text{Theoretical concentration}} \text{X } 100$$

2.7. Parallelism

Two FGM extracts were diluted serially 2-, 4- and 8-folds using the AA EIA's buffer. Percentage recovery was calculated similarly to dilution linearity. Parallelism was observed by evaluating relative accuracy using linear regression curves from the diluted samples and standard calibrators.

3. Results

3.1. FGM levels

We first aimed to compare the levels of FGM across the three immunoassays (Fig. 1). The quantification revealed that both the RIA (n = 144) and the CCC EIA (n = 70) had an overall lower mean (mean ± SEM: 64.1 ± 3.2 and 80.7 ± 4.1 ng/g, respectively) than the AA EIA (n = 141; mean ± SEM: 118.9 ± 6.4 pg/mL). Unfortunately, in our hands, our experience with the CCC EIA suggests that it is unreliable for our samples; in two different runs of 48 random samples, and after testing multiple dilution factors, all 48 samples in the two runs still gave values above the highest standard, precluding our ability to quantify their concentrations. This suggests that the CCC EIA may not be reliable for quantifying samples derived from yellow-bellied marmots under stressful conditions. Since we were unable to test these 48 samples within the CCC assay, they were excluded from all comparisons below, leaving the sample size from all three immunoassays as 70.

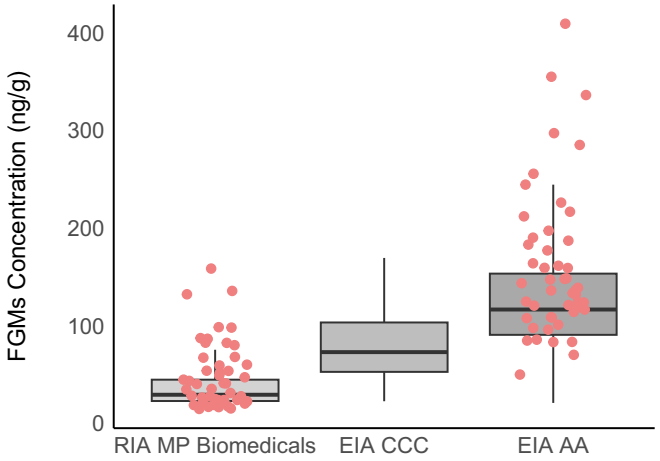

Fig. 1. Evaluation of yellow-bellied marmots' fecal glucocorticoid metabolites (FGM) using a radioimmunoassay (RIA; n = 144) or one of two competitive enzyme immunoassays (EIA) from either Cayman Chemical Company (CCC; n = 70) or Arbor Assays (AA; n = 141).
The dotted points are those samples that did not work in the EIA CCC in our hands.

3.2. Assay parameters

We next evaluated how each EIA performed across different analytical parameters, including the LoB, LLoD, LLoQ, ULoQ, and stability, using intra- and inter-assay CVs (Table 1), as well as TE and RE (Tables 2–3).

In our hands, the CCC EIA exhibited a large LoB, LLoD, and LLoQ due to variability in the zero and the lowest standard calibrators across different runs, as well as higher CVs, TEs and REs. However, the AA EIA had better overall metrics (lower LoB, LLoD, LLoQ, CVs, TEs and REs), suggesting that this immunoassay is more reliable for quantifying yellow-bellied marmot FGMs.

3.3. Correlational analysis

Previously, we successfully validated the use of the corticosterone RIA for the quantification of FGMs extracted from feces of yellow-bellied marmots (Smith et al., 2012). This validation was substantiated by observing a marked increase in the assay's FGM quantification in response to both biological and physiological stressors, including the ACTH challenge and live-trapping tests (Smith et al., 2012). Such findings indicate that the RIA method is sensitive and specific enough to detect genuine elevations in stress levels within yellow-bellied marmots. Due to constraints preventing the continued application of RIA, instead of validating the EIAs in a similar way, we sought to identify which EIA produced results with the greater correlation with the previously run RIAs.

We observed statistically significant moderate to strong correlations among the three immunoassays (Fig. 2A-C). However, the AA EIA showed the highest correlation with the RIA (Fig. 2B) for the same 70 samples that were run across all three immunoassays, suggesting it is the best choice for replacing the RIA for quantifying physiological stress levels in yellow-bellied marmots. When we added the rest of the samples that were measured in the RIA and AA, the correlation decreased (red line in Fig. 2B), but remained significant. Given that these samples failed in the CCC EIA, we are not able to include them as a part of the comparative correlation analysis. Although the correlation between CCC and AA was only slightly smaller than that between RIA and CCC, the correlation between RIA and CCC was substantially smaller for the same 70 samples that were run across all three immunoassays, suggesting that the CCC assay is not the best replacement for the RIA in our hands and for our purposes.

3.4. Covariate analysis

We next aimed to evaluate the influence of different random (ID and year) and fixed factors (sex and age) on FGM concentrations using the three immunoassays. We found no significant effects of sex or age on FGM concentration in any of the assays (Table 4).

3.5. Analytical validation of arbor assay EIA

Because the AA EIA had the best assay parameters and its sample concentrations were most closely associated with those of the RIA, we selected this EIA kit to further test its suitability with marmot fecal extracts (analytical validation) using spike recovery, dilution linearity and parallelism experiments (Taha, 2024) using two different fecal samples (undiluted concentration = 662.8 ± 0.0 and 381.1 ± 89.0 pg/mL). Most

Table 1
Analytical parameters for detection of fecal glucocorticoid metabolites (FGM) using the two competitive immunoassays. The limit of blank (LoB), lower limit of detection (LLoD), lower limit of quantification (LLoQ) and upper limit of quantification (ULoQ) are quantified as previously described (Armbruster and Pry, 2008; Dutta et al., 2023). CV – coefficient of variation; EIA – enzyme immunoassay.

Assay	Company	LoB (pg/mL)	LLoD (pg/mL)	LLoQ (pg/mL)	ULoQ (pg/mL)	Intra-assay CV	Inter-assay CV
Competitive EIA	Cayman Chemical Company	37.1	191.1	825.2	5797	16.3 %	16.3 %
Competitive EIA	Arbor Assays	22.1	27.9	70.9	5189	12.4 %	13.6 %

Table 2
Calculated concentrations, total error (TE) (Westgard et al., 1974), and relative error (Kat and Els, 2012) of fecal glucocorticoid metabolites (FGMs) using the competitive enzyme immunoassay (EIA) from Cayman's Chemical Company.

Corticosterone (pg/mL)	Assay 1		Assay 2		Assay 3		Assay 4		TE (%)	RE (%)
5000	4735	7175	4093	6677	4361	9625	4423	8998	113.16	25.22
2000	2024	2052	1949	1797	1919	1689	2028	1780	8.71	4.76
800	776.6	691.9	727.2	928	750.1	857.4	796.7	766.5	17.12	1.65
320	321.7	314.8	356	308.3	386.6	260.9	317.4	279.9	24.19	0.56
128	142.3	129.2	134	124.6	150.7	117.2	160.9	145.6	30.49	7.86
51	48.56	51.91	45.72	42.74	49.8	50.15	52.3	43.92	8.55	5.61
20	22.37	15.97	24.01	26.73	21.66	16.25	23.94	10.68	54.74	1.01
8	10.94	6.183	5.222	8.344	8.61	8.779	8.2	7.2	42.90	0.82

TE (%) = ((calculated concentration – actual concentration) + 2 SD)/actual concentration) X 100; RE (%) = ((calculated concentration – actual concentration)/actual concentration) X 100.

Table 3
Calculated concentrations, total error (TE) (Westgard et al., 1974), and relative error (Kat and Els, 2012) of fecal glucocorticoid metabolites (FGMs) using the competitive enzyme immunoassay (EIA) from Arbor Assays.

Corticosterone (pg/mL)	Assay 1		Assay 2		Assay 3		Assay 4		TE (%)	RE (%)
5000	4988	4710	5613	4653	4594	4734	4850	4796	10.34	2.66
2500	2650	2575	2541	2633	2655	2519	2749	2598	10.47	4.60
1250	1248	1248	1191	1166	1350	1271	1127	1251	9.57	1.48
625	593.7	615.5	570.5	729.8	611.4	622.1	590	697.6	18.39	0.61
312.5	326.9	309.6	338.2	276.4	317.8	298.2	271.8	357.8	18.79	0.13
156.25	165.3	151.2	138.9	199.9	152.1	139	138.2	169.9	27.46	0.36
78.1	87.91	73.18	52.76	89.08	86.11	99.04	68.71	88.95	41.30	3.35
39.05	41.13	27.03	41.53	32.04	31.94	35.94	25.6	58.87	48.75	5.86
19.5	33.02	12.25	22.89	23.49	21.74	18.53	18.05	19.3	69.66	8.51

TE (%) = ((calculated concentration – actual concentration) + 2 SD)/actual concentration) X 100; RE (%) = ((calculated concentration – actual concentration)/actual concentration) X 100.

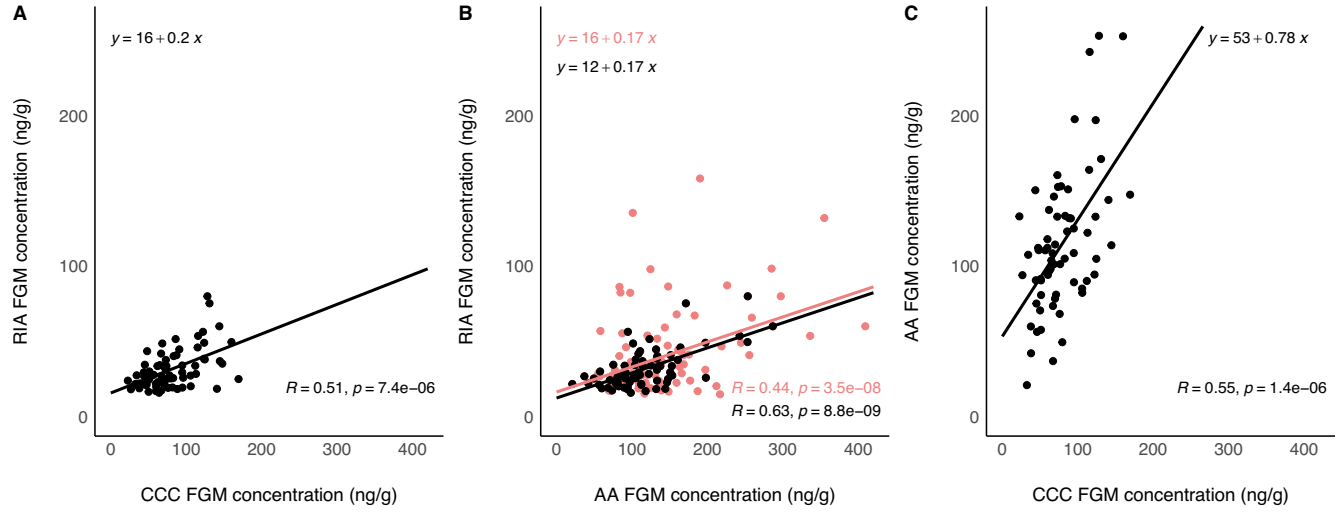

Fig. 2. A comparison of the association of FGM levels in yellow-bellied marmots' fecal samples as measured via its cross-reactions using a radioimmunoassay (RIA; n = 144) or two competitive enzyme immunoassays (EIA), one from Cayman Chemical Company (CCC; n = 70) and one from Arbor Assays (AA; n = 141). The black dots in panel B represent the same 70 samples that were run with the CCC and their correlation between the RIA and AA results. The highlighted dots are the additional 73 points that either failed or were never run using the CCC assay and their correlation between the RIA and the AA results. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Results for the linear mixed effects models illustrating a lack of interaction between the detected FGM levels in the RIA, CCC, and AA, and the covariates of sex and age. Year and ID were included as random factors.

	RIA (log **ng/g**)			**Cayman (log ng/g)**			**Arbor (log ng/g)**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	3.54	3.11–3.97	**<0.001**	4.36	4.14–4.57	**<0.001**	4.88	4.64–5.12	**<0.001**
sex [M]	0.06	−0.11–0.23	0.465	−0.16	−0.41–0.10	0.218	−0.10	−0.28–0.08	0.266
age	0.01	−0.02–0.05	0.450	0.01	−0.05–0.06	0.778	−0.01	−0.05–0.03	0.654

spike recovery (Fig. 3A), dilution linearity (Fig. 3B) and parallelism (Fig. 3C) experiments showed acceptable recoveries within the ±20.0 % range. Furthermore, we observed strong relative accuracy between the two diluted samples and the standard calibrator (Fig. 3D).

4. Discussion

We evaluated two corticosterone EIAs, that had been used for other rodents (Abelson et al., 2016; Eleftheriou et al., 2020; Morales et al., 2021), for their ability to measure FGMs (via their cross-reactions) from yellow-bellied marmot fecal extracts and for their association with past RIA results (using the same samples).

The AA EIA emerged as the superior method in our comparative analysis, consistently matching the RIA's accuracy across high and low FGM concentrations and proving to be the most reliable for detecting nuanced stress responses in yellow-bellied marmots (Fig. 1). The CCC EIA often exceeded the highest standard and could not reliably quantify high concentrations. In analyzing immunoassay parameters, the AA EIA outperformed the CCC EIA with higher sensitivity, and lower variability (Table 1) and error (Tables 2–3). Correlation analysis revealed strong associations between the AA EIA and the RIA, suggesting the AA EIA as the better replacement for the RIA (Fig. 2A-C). Although we note that the CCC and AA EIAs also are significantly correlated.

Overall, the AA showed superior assay parameters and robust analytical validation outcomes, which had no matrix effects (Fig. 3), and will be our method of choice for our ongoing longitudinal studies. We demonstrate that it is feasible for long-term longitudinal data sets that are using RIAs to switch to using an EIA for their new data; however, appropriate biological validations should accompany such a switch when possible. Furthermore, we suggest that an EIA can even perform better than an RIA. The best EIA kit to use will inevitably depend on the species and investigators should always test different EIAs. Here, we recommend use of the AA EIA over the CCC EIA for yellow-bellied marmots, and perhaps even over the RIA.

While the CCC EIA did not work well with our samples, other studies have documented its suitability with theirs. For instance, Hammond et al. (Hammond et al., 2019) showed that the CCC EIA was suitable for quantifying FGMs in California ground squirrels (*Otospermophilus beecheyi*). A possible reason for this discrepancy could be due to different methodologies between our group and theirs in isolating FGMs or,

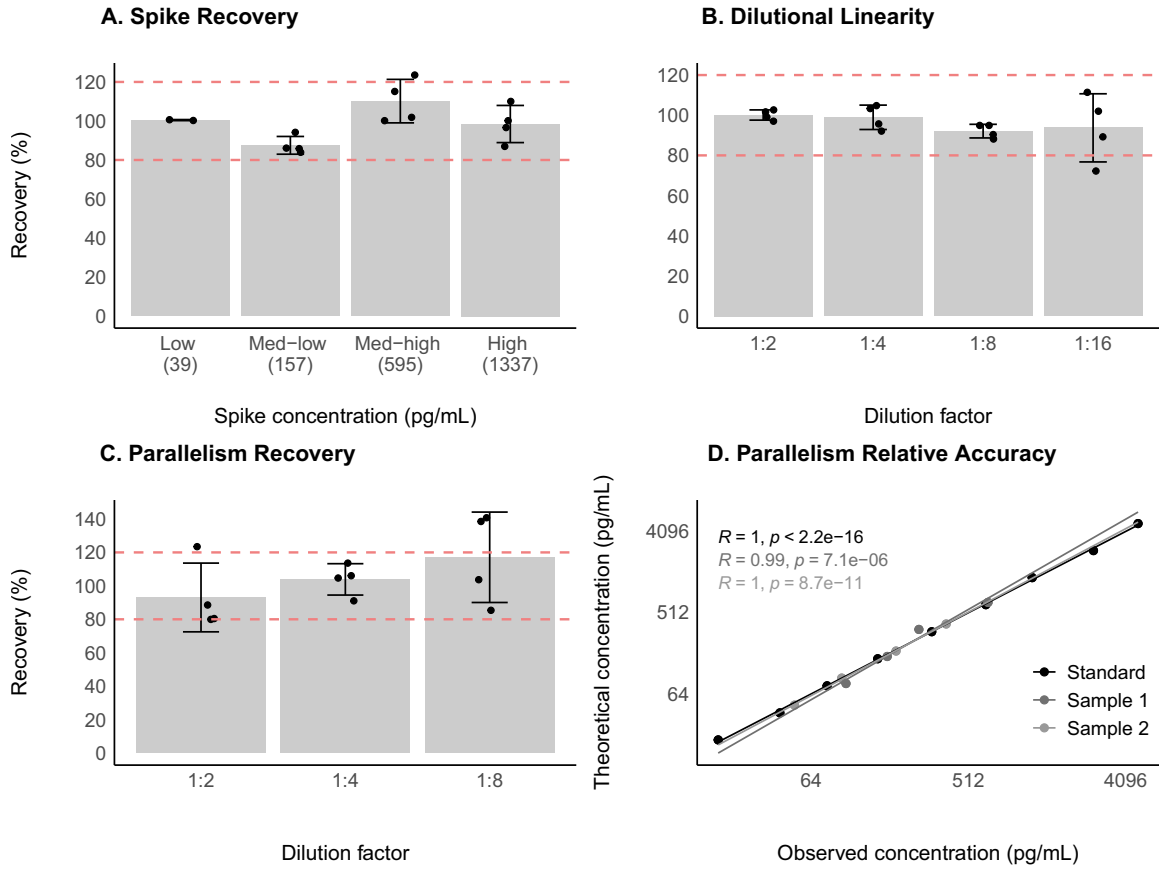

Fig. 3. Analytical validation of the competitive enzyme immunoassay (EIA) from Arbor Assays for quantification of FGMs in yellow-bellied marmot fecal extracts using spike recovery **(A)**, dilutional linearity **(B)** and parallelism **(C, D)**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

perhaps more likely, a difference in the FGMs themselves. In fact, it must be emphasized that corticosterone immunoassay kits are designed to measure corticosterone not FGMs, which may vary in their composition across species. Given that a corticosterone RIA had already been biologically validated for FGMs in yellow-bellied marmots, we assumed that yellow-bellied marmot FGMs are similar-enough to corticosterone to allow other corticosterone immunoassays to detect changes in stress. Clearly the kits vary in their ability to do so, even though both the CCC and AA kits had previously been validated for FGMs in other rodent species (Abelson et al., 2016; Eleftheriou et al., 2020; Morales et al., 2021). While our study was limited in time and funding, ideally one should perform both analytical and biological validations across different available assays that are capable of detecting FGMs and we encourage others to do so.

5. Conclusions

We conclude that the AA EIA is an appropriate kit to use when evaluating FGMs in wild yellow-bellied marmots. The immunoassay showed a moderate association with the RIA kit for detecting FGMs across sex and age, even in samples that had been extracted 7 years previously. We also analytically validated this immunoassay as one satisfactory method of quantifying FGMs in yellow-bellied marmots, showcasing the lack of matrix effects with the AA EIA.

Finally, we demonstrate that long-term datasets can switch from RIA to EIA, but we urge future developments to keep longitudinal datasets in mind, maintain access and support for older immunoassays, and clearly communicate any changes. Moving away from RIAs will require better access to a wide variety of EIAs, including those that are designed to specifically detect FGMs rather than the native GCs.

Author contributions

HBT, XOR and DTB conceptualized and designed the study. XOR curated the data, managed the project, and created the figures. XOR, EP, and HBT performed the experiments and collected the data. XOR and HBT analyzed the data. HBT, XOR and SR wrote the manuscript. DTB provided data, funding, discussed results, edited and approved the final manuscript draft.

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Data sharing

All data and code has been posted in a public OSF repository at https://osf.io/umqvn/ DOI: 10.17605/OSF.IO/UMQVN

CRediT authorship contribution statement

Xochitl Ortiz-Ross: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hash Brown Taha:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Emily Press:** Investigation. **Sarah Rhone:** Writing – original draft. **Daniel T. Blumstein:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

The authors have no conflicts to declare.

Data availability

The data has been uploaded to OSF and is included in the article

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