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Migrant blackbirds (*Turdus merula*) on stopover have higher plasma PYY compared to residents: A validation and proof-of-concept

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ABSTRACT

Migratory birds expend substantial energy during flight and often rely on stopovers to refuel. Foraging behavior during stopover might vary with fuel loss and food availability though likely involves higher food intake rates than in non-migratory (resident) individuals. In mammals, hormones such as leptin signal information about energy balance and satiety. In contrast the gut hormone peptide YY (PYY), which rises rapidly after feeding and suppresses appetite in mammals, might serve a similar functional role in songbirds, which lack functional leptin. We validated and measured plasma PYY in migrant and resident Common Blackbirds (*Turdus merula*) on Helgoland, a key North Sea stopover site, during peak autumn migration. Migrants exhibited significantly higher circulating PYY levels than residents, possibly reflecting increased or differential foraging. PYY was unrelated to body condition or fat stores. This proof of concept study justifies future work on plasma PYY and other metabolic signals and their potential influence on avian migratory decision making.

1. Introduction

Most migratory birds must replenish energy stores during stopovers, and decisions about when to resume migration likely depend, in part, on foraging success during these periods. However, the physiological mechanisms underlying migratory decision-making remain unresolved (Watts and Cornelius, 2023), partly due to species differences in migratory strategy and local ecological conditions. A key physiological factor influencing these decisions is the rate of energy accumulation, largely through fat deposition (Alerstam, 2011). Body condition has been widely studied (Fusani et al., 2009; Eikenaar and Bairlein, 2014), but quantifying foraging success during stopover is rarely feasible in free-living birds (Guglielmo, 2018). Here, we test whether peptide YY (PYY)—a gut-derived hormone that rises after feeding in mammals—is associated with migratory status in Common blackbirds (*Turdus merula*).

Appetite regulation involves endocrine signals acting on different time scales. Ghrelin, a gut-derived orexigenic peptide, rises in anticipation of meals and stimulates feeding on relatively short time horizons. In contrast, leptin is secreted primarily by adipocytes and reflects longer-term energy stores; although it can suppress food intake, it does not function as an acute postprandial signal and is not a gut-derived satiety peptide (Friedman-Einat and Seroussi, 2019). These distinctions are important in passerine birds, where functional leptin appears absent and ghrelin's role is still unresolved, motivating investigation into alternative short-term appetite signals (Prost et al., 2025). In mammals, PYY is released from intestinal L-cells within minutes after nutrient ingestion, peaks at roughly one hour, engages hypothalamic satiety pathways, and remains elevated for several hours (Batterham et al., 2002). Although its function in birds remains poorly characterized, studies in teleosts and poultry suggest a conserved role in postprandial satiety (Aoki et al., 2017; Reid et al., 2017; Assan et al., 2021). If avian PYY reflects recent food intake, it may offer a minimally invasive indicator of foraging success during stopover.

The Common blackbird (*Turdus merula*) is an ideal species for investigating migratory physiology, as it exhibits diverse migratory strategies across populations (Linek et al., 2021). While some

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individuals are fully migratory, others remain resident year-round, and some populations contain both types. This variation permits direct comparison of physiological traits associated with migratory versus non-migratory states within the same species.

We studied blackbirds on Helgoland, a small island in the North Sea that serves as a key stopover site during autumn migration and also supports a stable population of resident birds. In this proof-of-concept study we conducted technical validations for plasma PYY levels in blackbirds. We then compared circulating PYY levels in concurrently captured resident and migrant birds and found that migrants had elevated levels, possibly owing to differential feeding. Combining this metabolite with others could offer additional mechanistic targets for understanding how feeding is linked to migration.

2. Materials and methods

2.1. Animal capture, measurement and sampling

We captured and bled blackbirds ($N = 98$; 80 migrants, 18 residents) on the island of Helgoland during autumn migration, from 14th October to 13th November 2019, in the trapping garden of the Institute of Avian Research island station on Helgoland (54°11′N, 07°55′E). Birds were captured and sampled hourly between 0830 and 1830. Traps consist of fruit gardens that narrow into a terminal enclosure box. Birds are swept hourly from the garden into the enclosure and then individually held briefly in cloth bags. We collected 100–200 μL of whole blood by alar vein puncture and centrifuged at 5000*g* at 4 °C for 20 min to separate plasma. The plasma was stored at −20 °C until transported to the mainland where it was stored at —80C from 2019 to 2025, at which point they were shipped on dry ice to the Max Planck Institute for Animal Behavior and maintained at —80C for 3 days before being analyzed. For each bird, fat and lean mass were measured with quantitative magnetic resonance (QMR) using an EchoMRI™ whole-body scanner (EchoMRI Body Composition Analyser E26–262-BH, Zinsser Analytic GmbH, Frankfurt am Main, Germany; see Kelsey et al., 2019).

The sampled birds were assigned as migrants or residents: On Helgoland, Common blackbirds are caught throughout the year. Birds being caught outside of the migratory season (mostly during the breeding season) are ringed with a colour metal ring. Therefore, during the migratory season, these resident birds could be identified by the rings. Birds without a ring were assigned as being migrants (Eikenaar et al., 2015).

2.2. Enzyme immunoassays

We used a commercial enzyme immunoassay kit to measure avian plasma PYY (Cusabio Cat. No. CSB-EL019128CH, Wuhan, Hubei, China). The kit reports <15% CV for both intra- and inter-assay precision and a range of 90–97% recovery in chicken plasma. Prior to use we validated the assay for use with blackbird plasma (SM1). For each sample, we subsampled 5 μL of micro-vortexed plasma and diluted it in 125 μL of RO water (1:26 dilution; SM1) and plated 50 μL of sample in duplicate wells in 96 well plates along with six internal standards, blank wells and three external standards per plate consisting of a pool of blackbird plasma used to determine intra- and inter-assay coefficients of variation. We then added conjugate to all wells (except blanks). After 1 h at 37C (Melag incubator) we then washed wells with wash buffer (kit-supplied) three times and incubated with 50 μL of horseradish peroxidase-avidin (except blanks) for 30 min at 37C. We then repeated the wash procedure three times and added 50 μL of the two substrates and developed for 10 min at 37C in darkness. Then we stopped the reaction by adding 50 μL of stop solution and determined the optical density with a microplate reader (BioRad iMark 100, Berkeley, CA, USA) at 450 nm. After each addition to the plate (except stop solution) we placed the plate on an orbital shaker (NeoLab, Heidelberg, Germany) at 500 rpm for 15 s to ensure thorough mixing. We confirmed two very high PYY values by re-assaying diluted aliquots (optimal dilutions 1:52 and 1:200).

2.3. Statistics

All analyses were conducted in R (version 4.3.2; R Core Team, 2023). To examine the effect of predictors on PYY concentration, we fitted a Bayesian robust linear model with a Student-t likelihood using the brms package (Bürkner, 2017). The response variable was PYY ($\log_{10}$-transformed) and "strategy" (2 levels: migrants/residents), "sex" (2 levels: female/male) and "age" (2 levels: juvenile/adult) as fixed factors and fat stores (QMR values), the time of the day (Migrants: median = 10:30, range = 08:30–17:30; Residents: median = 11:15, range = 08:05–18:08) and julian day as covariates. We used weakly informative priors: a normal (0,1) prior on all regression coefficients and a Student-t (3, 0, 2.5) prior on the residual standard deviation. The model was run with four chains and 4000 iterations per chain (2000 warmup). Convergence was assessed using trace plots, effective sample sizes and using the Gelman-Rubin convergence diagnostic (Rhat = 1). We defined effects as statistically significant when the 95% credible intervals did not overlap zero. All continuous predictors were mean-centred and scaled (z-transformed) prior to analysis. No model selection was performed and we retained all main effects. An outlier PYY value (migrant) was identified (z-score; $p < 0.05$) and was included or excluded to evaluate the robustness of the results. We complemented this Bayesian approach with a simple nonparametric Mann-Whitney *U* test as well as a logistic regression to evaluate the simple relationship between PYY concentrations and migratory status. Effect sizes were calculated as standardised mean differences (Cohen's d).

3. Results

Blackbird plasma demonstrated reliability and precision using the commercial avian PYY EIA kits (see SM1). Dilutions of pooled blackbird plasma were parallel to the standard curve with an optimal dilution of 1:26, which permitted the use of 5 uL of plasma for most samples. Coefficients of variation were low (intra-assay = 2.9%; inter-assay = 7.2%) and technical repeatability was high ($R^2 = 0.7$).

Plasma PYY concentrations were higher in migrants than residents ($\beta = -0.60$, 95% CI [−0.75, −0.46]; Cohen's d = 0.60; Fig. 1). Age showed a weak positive effect ($\beta = 0.12$, 95% CI [0.01, 0.22]), while the estimates for sex, fat stores, time of day, and Julian day included zero, indicating a lack of evidence for effects of these predictors (SM2). The results of a conventional Mann-Whitney *U* test and logistic regression likewise yielded significantly elevated PYY levels in migrants (SM3). Although the sample variance of PYY was markedly higher in migrants (variance ratio ≈ 148; SM4), this difference was not statistically significant with a median-centered Levene's test ($W = 0.23$, $p = 0.63$), including when the largest one or two migrant outliers were removed (all $p > 0.14$). Because sample sizes were highly unequal ($N = 80$ vs. 18), this test has limited power to detect variance heterogeneity.

4. Discussion

Here we provide the first proof of concept demonstration that plasma PYY can be reliably measured in a passerine. Our validation results indicate that blackbird plasma PYY levels are enriched and small plasma samples are sufficient to precisely measure concentrations in the nanogram per milliliter range. Further, PYY concentrations behaved in a proportional and predictable manner using an antibody generated from chicken. Lastly, individual samples showed high repeatability across duplicate measurements and exhibited low coefficients of variation. These validations provide an informed basis for future passerine plasma PYY studies.

Migrating blackbirds captured on Helgoland during autumn exhibited significantly higher circulating PYY levels than resident conspecifics.

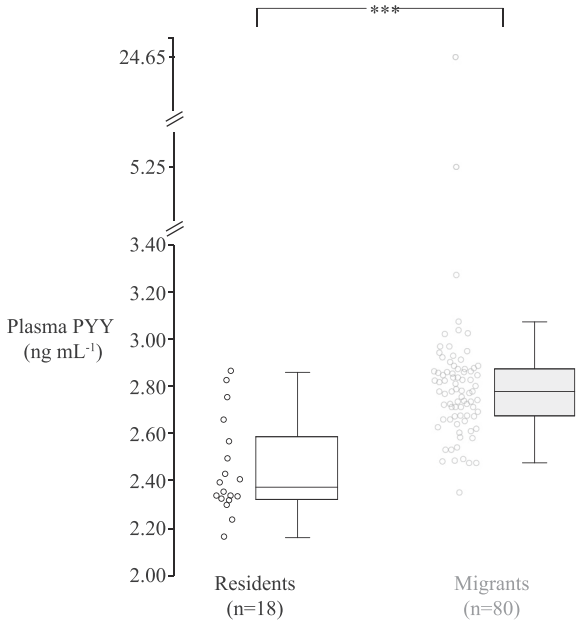

Fig. 1. Plasma PYY concentrations in resident and migrant (during stopover) Common Blackbirds (*Turdus merula*) on Helgoland island (Germany). Box and whisker plots are accompanied by individual bird data points. Migrants had higher PYY concentrations (*** $p < 0.001$), with or without the high outlier (migrant).

If PYY in birds mirrors its function in mammals—rising rapidly and proportionally with food intake (Batterham et al., 2002; Reid et al., 2017)—this pattern could reflect increased or divergent foraging activity among migrants. To our knowledge this is the first study of plasma PYY in wild birds. It should be noted that the sequence for PYY in blackbirds is not currently known, but given the high homology of PYY in other birds (*Gallus gallus*: XM_015273299, Conlon and O'Harte, 1992; *Coturnix japonica:* NC_029542.1; *Passer montanus:* XP_039556608; Unpublished data) we assume that the antibody raised against chicken binds the corresponding peptide in blackbirds.

Because arrival time on the island for the migrants was technically unknown (though assumed to be very recent; Eikenaar et al., 2015, 2018), PYY variability may reflect arrival recency, with newly arrived migrants still locating suitable foraging sites. Further, differences in PYY levels between residents and migrants might reflect variation in nutritional content of food, with proteins and fats more likely to deliver undigested or partially digested nutrients to the distal gut, where they can activate nutrient-sensing receptors on L-cells and thereby promote PYY (Batterham et al., 2002). A previous study of Blackbirds on Helgoland indicated that migrants exhibit much higher plasma levels of an essential fatty acid (alpha-linoleic acid) compared to residents, indicating a diet likely enriched in seeds or terrestrial invertebrates (Jensen et al., 2020). Future studies will benefit from performing plasma metabolite panels that include PYY, as refueling birds are known to exhibit rapid changes in circulating levels of triglycerides and β-hydroxybutyrate–two reliable proxies for energy balance and recent food intake, and which are often measured to evaluate differences in stopover site quality or foraging abilities (Jenni-Eiermann and Jenni, 1994; Guglielmo et al., 2005; Zajac et al., 2006). A small subset of migrants (~3%) exhibited exceptionally high PYY levels. Understanding the behavioral context of these individuals warrants further investigation; for example, whether they are particularly effective foragers, how this relates to subsequent changes in body condition and what, if any, relationships there are to movement decisions. No resident birds showed similarly elevated PYY levels. Relatedly, we know that captive blackbirds across multiple populations that are fed ad libitum exhibit consistently lower and less variable PYY concentrations compared to wild caught birds (unpublished data). Extreme PYY levels might also reflect altered downstream feedback processes, as appetite-suppressants might be down-regulated to support hyperphagia and migratory fattening (Cornelius et al., 2013).

Although we did not assess individual movement or refueling behavior, our results suggest that elevated PYY in migrants reflects an altered foraging regime or refueling physiology or both. Future studies could test whether gut-derived signals like PYY vary with migratory decision making. In Common blackbirds, metabolic and endocrine factors are known to covary with migratory strategy (Twining et al., 2023; Eikenaar et al., 2018), suggesting that peptides involved in appetite regulation could also shift with changes in energetic demand. The lack of an association between PYY and fat content measured by QMR further suggests that PYY may reflect aspects of internal state—such as gut activity, short-term satiety signaling, or transient energy balance—that are not captured by more stable fat stores. These findings underscore the need for targeted work incorporating foraging data or refueling biomarkers (e.g., plasma triglycerides, ketones, mass change) to determine how PYY relates to feeding intensity and migratory physiology in free-living songbirds.

Finally, the role of diet composition in modulating PYY remains to be explored. In mammals, PYY release varies with macronutrient content, with high-protein meals often eliciting stronger responses (Woodward et al., 2022). Given that exogenous PYY suppresses appetite in mammals (Batterham et al., 2002), diet quality during stopovers may influence both circulating PYY and migratory decisions. Because songbirds lack functional leptin (Friedman-Einat and Seroussi, 2019; Prost et al., 2025), PYY may partially compensate and contribute to migratory decision making. Future work should explore feeding intensity, nutrient content (Gao et al., 2024), and metabolic status to clarify how nutritional context shapes PYY dynamics and migration decisions.

CRediT authorship contribution statement

Alexander T. Baugh: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jesko Partecke:** Writing – review & editing, Resources, Funding acquisition. **Natalie Wellbrock:** Writing – review & editing, Investigation. **Roberto Carlos Frias-Soler:** Writing – review & editing, Investigation. **Franz Bairlein:** Writing – review & editing, Investigation. **Alba Hykollari:** Writing – review & editing, Validation. **Leonida Fusani:** Writing – review & editing, Methodology. **Pablo Salmón:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics

Trapping, ringing and handling of the birds complied with the law of the Federal State of Schleswig-Holstein, Germany. The permit to sample Common blackbirds on Helgoland was granted by the Ministerium für Energiewende, Landwirtschaft, Umwelt, Natur und Digitalisierung, Schleswig-Holstein, Germany (V 244–9730/2019).

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Declaration of competing interest

We have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2026.105885.

Data availability

The complete dataset is made available in Electronic Supplemental Materials (SM5).

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