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## SYMPOSIUM

# Detecting Bias in Large-Scale Comparative Analyses: Methods for Expanding the Scope of Hypothesis-Testing with HormoneBase

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**Synopsis** To address large-scale questions in evolutionary biology, the compilation of data from a variety of sources is often required. This is a major challenge in the development of databases in organismal biology. Here, we describe the procedure we used to reconstruct the phylogeny of the 474 species represented in HormoneBase, including fish, amphibians, mammals, birds, and reptiles. We also provide the methodology used to compile vertebrate environmental, life history, and metabolic rate data for use in conjunction with the HormoneBase database to test hypotheses of the evolution of steroid hormone traits. We then report a series of analyses using these data to determine the extent to which field measures of circulating hormones and associated life history data exhibit taxonomic and geographic bias. By providing a detailed description of the approaches used to compile and evaluate these data and identifying potential biases in the collection of these data, we hope to make the HormoneBase database a more broadly useful resource for the scientific community to address a diversity of comparative questions.

## Introduction

Many empirical questions in biology require the compilation of data from multiple sources, yet for large-scale analyses, this can be an enormous task. While molecular biologists have had established procedures for compiling nucleotide and protein sequence data in standardized formats for decades (e.g., GenBank, [Benson et al. 2013](#)), only in recent years have organismal biologists begun developing large, multi-species databases of ecological, morphological, behavioral, and life history traits. Examples of such databases include [Lislevand et al. \(2007\)](#), who provide a database of avian body size and mating systems; PanTHERIA, which provides life history, ecology, and geography of extant and extinct mammals ([Jones et al. 2009](#)); [Myhrvold et al. \(2015\)](#), who provide life history data on amniotes; and COMADRE, a database of animal demography traits

([Salguero-Gómez et al. 2016](#)). Any one database has limitations, however, and the data needed to directly test many comparative hypotheses, including a phylogeny of the taxa included in the database, may require information not available in a given database.

To address questions on the evolution of hormones and their variation, [Vitousek et al. \(2018\)](#) created HormoneBase ([hormonebase.org](http://hormonebase.org)), a database that includes more than 6580 measures of plasma androgens and glucocorticoids from 474 species of free-living, unmanipulated, adult vertebrates. In brief, HormoneBase provides mean, variation, and range of androgens and glucocorticoids (including baseline and stress-induced measures), as well as sex, month, and year of study, geographic coordinates and elevation, life history stage, method and latency of hormone sampling, and the hormone

analysis techniques associated with the hormone measurements.

Here, we present our approach to reconstructing a phylogeny for the taxa represented in the database, in order to enable phylogenetically-controlled analyses. The HormoneBase database, together with this phylogeny, provides a valuable tool for comparative analyses of hormonal evolution. We also provide detailed methodology describing how researchers compiled additional data on life history traits, metabolic rate, and the environment in which study populations occurred, to test specific hypotheses about the evolution of steroid hormone levels across taxa. We then test a series of hypotheses to determine the extent of taxonomic and geographic biases in available hormone data to reveal meaningful opportunities for the focus of future work.

## Reconstruction of phylogenetic tree

Any multi-species comparison in modern biology requires phylogenetic information on the evolutionary relationships among the species under consideration. Most phylogenetic comparative analyses require not only the topography of a resolved phylogeny, but also the relative lengths of each branch in the tree. While robust phylogenies are available for most vertebrate groups, combining those separate lineage-specific trees into a single tree that includes all 474 species in HormoneBase was a non-trivial task.

To construct a fully-resolved, species-level phylogeny of the species in HormoneBase (Fig. 1), we began with a time-dated backbone phylogeny from the TimeTree of Life (Kumar et al. 2017). This backbone included one tip for each of the major animal lineages represented in HormoneBase: *Petromyzon marinus* (lampreys, Petromyzontiformes), *Acipenser stellatus* (ray-finned fishes, Actinopterygii), *Ambystoma maculatum* (amphibians, Amphibia), *Tachyglossus aculeatus* (mammals, Mammalia), *Sphenodon punctatus* (squamates and the tuatara, Lepidosauria), *Chelydra serpentina* (turtles, Testudines), *Alligator mississippiensis* (crocodiles, Crocodylia), and *Meleagris gallopavo* (birds, Aves). We manually modified the date of the amphibian stem node by shifting it to be in accord with Roelants et al. (2007), while ensuring the tree remained ultrametric. The TimeTree of Life does not include a stem date for the Chondrichthyes, so we based our estimate on the date in the online Tree of Life Explorer (Rosindell and Harmon 2012), and created a branch leading to sharks by binding in a tip for *Rhizoprionodon taylori* (Revell 2012). Based on this backbone tree, we then manually grafted the remaining shark ( $n = 3$ ) and

lamprey ( $n = 1$ ) species into the tree, recreating the topology and divergence times in the TimeTree of Life and Tree of Life.

We then matched taxonomy between HormoneBase and major lineage-specific trees (ray-finned fishes [Rabosky et al. 2013], amphibians [Pyron and Wiens 2011; Eastman et al. 2013], mammals [Bininda-Emonds et al. 2007], squamates [Pyron et al. 2013], turtles [Jaffe et al. 2011], and birds [Jetz et al. 2012]), such that each row in the database matched one and only one tip in a lineage-specific tree. We employed 12 phylogenetic equivalent tip-row swaps, *sensu* Pennell et al. (2016). The mammal tree was not completely resolved, so we employed the R (R Development Core Team 2016) package *ape* (Paradis et al. 2004) to randomly resolve the few polytomies in our reduced mammal tree. Similarly, the fish tree was not ultrametric according to the tolerance threshold of R, so we used functions in *phangorn* (Schliep 2011) to force the tree to be ultrametric (the differences in branch lengths between the input and final, ultrametric tree were extremely small). Once these lineage-specific subtrees were pruned to match the species in HormoneBase (or tips were swapped out of the subtrees to match the phylogenetic equivalent in HormoneBase), we bound these into the backbone tree using a simple custom workflow. In practice, we found the crown age of the remaining taxa in each subtree, removed that much of the stem lineage in the backbone tree, and then bound the subtree into the backbone terminal for the relevant lineage. This resulted in a fully-resolved, ultrametric tree where each row in HormoneBase matched to a single species in the phylogeny (phylogeny available as Supplementary Data).

## Additional data for comparative analyses using HormoneBase

There are many types of data that can complement the hormone measures compiled in HormoneBase, allowing for a wide range of comparative analyses that utilize the data available in HormoneBase. Thus, we compiled additional data on the populations and species represented in HormoneBase. Here, we use these data to probe where and to what extent the measures in HormoneBase reflect taxonomic or geographical bias in sampling for hormone and life history traits.

For examples of large-scale comparative analyses that utilize these types of data in testing hypotheses of hormonal evolution using HormoneBase, see Casagrande et al. (2018, this issue) for an analysis of the evolution of life history and glucocorticoids in

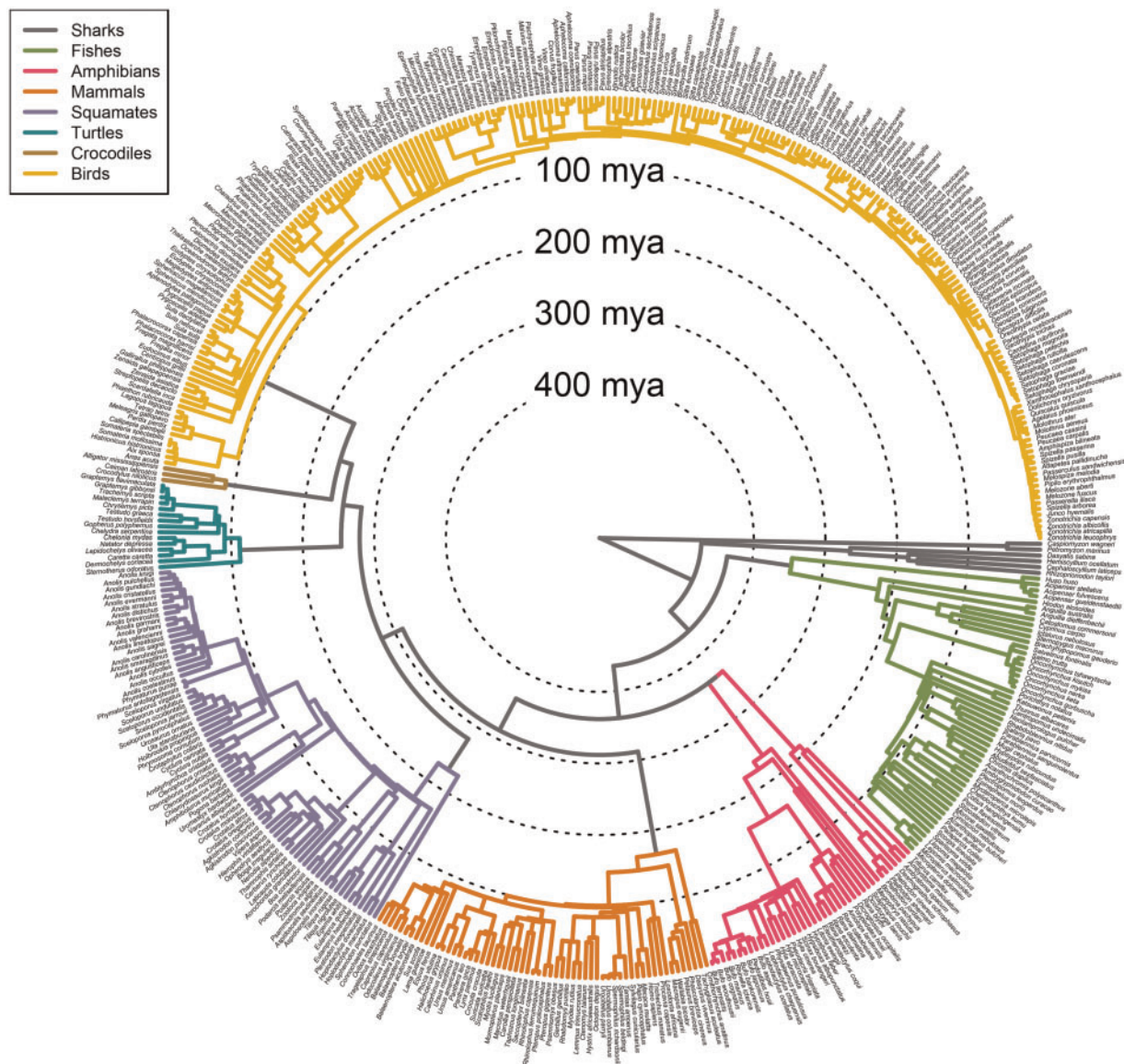


Fig. 1 Phylogeny of vertebrate taxa included in HormoneBase.

birds, Francis et al. (2018, this issue) for an analysis of metabolic scaling and glucocorticoids, and M. N. Vitousek et al. (in preparation, will be submitted for review by June 2018) for an analysis of the relationships among glucocorticoids and environmental, metabolic rate, and life history traits across tetrapods.

### Extraction of environmental data

Short- and long-term patterns of temperature and precipitation, especially severe weather events, can directly influence an organism's physiological state, and can ultimately affect survival and reproductive fitness (reviewed in Wingfield and Sapolsky 2003). If weather is a cause of stress, glucocorticoid levels may

vary in response to both predictable and unpredictable events such as storms, drought, or heat waves (e.g., Romero et al. 2000). Further, short- and long-term weather patterns can influence an organism's developmental trajectory, dominance relationships, or reproductive investment, all of which may be associated with variation within and among individuals in androgen and other sex-steroid hormone levels (e.g., Gombe and Oduor-Okelo 1977; Wingfield et al. 1983). However, it is not yet known whether large-scale environmental patterns have consistent influences on organisms across broad taxonomic scales. Thus, to explore the environmental correlates of hormonal variation within and among groups at any level of taxonomic organization, we compiled a series of measures of environmental variation.



In addition, temperature and precipitation measures associated with particular localities (and thus the populations that occur in those localities) can also reflect the range of environmental conditions in which hormone data are measured. If hormone sampling is underrepresented in particular habitats across the globe, studies of the relationships between hormonal traits and environment may be limited in power and interpretation.

In our compilation of hormone measures in HormoneBase (Vitousek et al. 2018), we recorded the month and year of data collection, and the latitude, longitude, and elevation for each population. If latitude/longitude and elevation data were provided in the original report of the hormone measures, we used the authors' reports after confirming that they were correct (i.e., concordant with the description of the location of hormone data provided in the paper) using Google Earth. If the locality of hormone data collection was described in the publication, but latitude and longitude were not provided, we used Google Earth to estimate latitude and longitude for that locality. If elevation was not provided in the publication, we used latitude and longitude to estimate elevation via Google Earth or GPSO (<https://www.geoplaner.com/>).

For each locality in which hormone data were measured, we obtained precipitation, temperature, and potential evapotranspiration (PET) data for all years in which hormones were measured in the field (i.e., 1965–2015). PET is a measure that quantifies an environment's potential moisture deficit by integrating radiation, temperature, and humidity (Fisher et al. 2011). We acquired these data from the CRU-TS 4.0 Climate Database (Harris et al. 2014), which provides monthly global land cover data at a resolution of  $0.5 \times 0.5$  degree grid cells. Specifically, temperature values reflected the monthly average of daily mean temperature in degrees Celsius, precipitation reflected cumulative millimeters per month, and PET reflected monthly average of millimeters per day. We chose to use these data because they are among the best relatively fine-scale global climate data that include the time series necessary for HormoneBase and because they have been used for several large-scale biological analyses (Garcia et al. 2014; Stephens et al. 2016; Siepielski et al. 2017). We also obtained cumulative monthly net primary productivity (NPP) at the same grid resolution for years 2000–2010 from NASA's Moderate Resolution Imaging Spectroradiometer. For each study location, for all climate data except NPP, we obtained all monthly values spanning 1965–2015 using the extract function in the Raster 2.6 R package (Hijmans and van Etten 2014). For NPP, this was restricted to

data availability, i.e., 2000–2010. From these data, it is then possible to calculate annual or seasonal averages or measures of variation to describe an environment at multiple time scales.

### Compilation of life history traits

To test our focal hypotheses on hormonal evolution, we required a number of measures of life history traits. For example, circulating levels of androgens and glucocorticoids may vary dramatically across body size, life history stage, and pace of life, among many other factors. Yet, all of these data are rarely provided in published reports of hormone measures, and there is currently no one source of life history traits that provides comparable data for all of the species in our database.

We focused our compilation of life history data on variables such as body size of each sex (reported as body mass), lifespan, reproductive attempts per year, and metabolic rate. We compiled these data from a variety of sources. When population-specific information was provided in the articles that reported measures of hormones reported in the HormoneBase database, we used those data. We also searched the primary scientific literature for additional information (including the amniote database provided by Myhrvold et al. 2015), and used reputable online sources such as the Animal Diversity Web (<https://animaldiversity.org/>; Myers et al. 2018), Encyclopedia of Life (<http://eol.org/>; Parr et al. 2014), AnAge (<http://genomics.senescence.info/species/>; de Magalhães et al. 2005), FishBase (<http://www.fishbase.org/>; Froese and Pauly 2018), AmphibiaWeb (<https://amphibiaweb.org/>; AmphibiaWeb 2018), Birds of North America (<https://birdsna.org/>; Rodewald 2015), and the Handbook of Birds of the World (<https://www.hbw.com/>; del Hoyo et al. 2018).

Life history data were not equally available across species. Whenever possible, we focused on compiling data from the specific population of study in which hormones had been measured. When we were not able to locate population-specific data, we gathered species-specific data. When species-specific data were not available, we occasionally used data from closely-related and ecologically-similar congeners. Biologists who were experts in each taxonomic group compiled the data for each group.

### Compilation and standardization of metabolic rate data

The energetic costs associated with survival and reproduction result in remarkable variation in rates of metabolism across animal taxa (reviewed in Burton et al. 2011). In both natural populations and

experimental studies of vertebrates, variation in metabolic rates is often associated with variation in steroid hormone levels (e.g., birds: Wikelski et al. 1999; Buchanan et al. 2001; amphibians: Wack et al. 2012; reptiles: Miles et al. 2007; DuRant et al. 2008; mammals: Haase et al. 2016). Results from such studies have shown that variation in energy expenditure can alter levels of circulating hormones, and variation in hormonal levels can affect metabolic rates. Thus, one of our goals with the HormoneBase data was to explore relationships between metabolic rate and hormonal variation across vertebrate taxa.

We compiled data on whole animal metabolic rates from the primary literature and several existing reviews (White et al. 2006; Makarieva et al. 2008; Sieg et al. 2009; Londoño et al. 2015; Stager et al. 2016; Uyeda et al. 2017). Following Gessaman and Nagy (1988), we converted  $\text{VO}_2$  consumed to heat production using a conversion factor of 20.1 J/ml  $\text{O}_2$  and a respiratory quotient (RQ) of 0.72; where  $\text{VCO}_2$  was presented, it was converted using a factor of 27.3 J/ml  $\text{CO}_2$  which assumes an RQ of 0.72. These measures of heat conversion were then converted into watts, to enable comparison of metabolic rates across species. When mass specific metabolic rates were presented, they were multiplied by body mass to obtain whole animal metabolic rates.

The data we compiled for endotherms (birds and mammals) were basal metabolic rates (BMRs) and the data we compiled for ectotherms (fish, amphibians, and reptiles [i.e., squamates, turtles, and crocodilians]) were standard metabolic rates (SMRs). These measures each represent the minimal energy needed to maintain normal organismal function in a post-absorptive, inactive animal during the active phase of their daily cycle (Fry 1971; McNab 1997). For endotherms, these conditions occur when the environmental temperature experienced by the animals is within their thermal neutral zone, as is required for BMR measurements. The metabolic rate of ectotherms increases with environmental temperature, so SMR is a comparable measure of BMR if all measurements of SMR are made temperature independent (White et al. 2006). Thus, SMR data were temperature-corrected.

We followed Downs et al. (2008) to correct SMR data to a standard temperature. This approach is rooted in a multiple regression approach based on a Boltzmann–Arrhenius approach to correct for body temperature, rather than a  $Q_{10}$  approach (Gillooly et al. 2001; Downs et al. 2008). Previous analyses of large metabolic rate datasets indicate that either approach yields similar results (White et al. 2006; Uyeda et al. 2017). Specifically, we used all of the data for fish, reptiles, and amphibians compiled by

White et al. (2006) and supplemented by additional data from the literature to perform independent linear regression models for each major taxonomic group, following the convention of other studies (Gillooly et al. 2001; Nagy 2005; White et al. 2006; Downs et al. 2008). We used the whole supplemented datasets from White et al. (2006) because a larger dataset gives a better estimate of the mean relationship between temperature and SMR. We used the `lm` procedure in R (R Development Core Team 2016) to fit the following linear model:

$$\ln B = \ln a + c(1000/T). \quad (1)$$

In this equation,  $B$  is SMR,  $a$  is the intercept, and  $T$  is the environmental temperature at which the SMR measurement was collected in  $K$ . If a researcher was interested,  $c$  could be used to back-calculate the specific values of the Boltzmann–Arrhenius model (Downs et al. 2008).

Our dataset included data for 64 fish species, 146 amphibian species, and 159 reptile species. The taxonomic group-specific regressions were as follows:

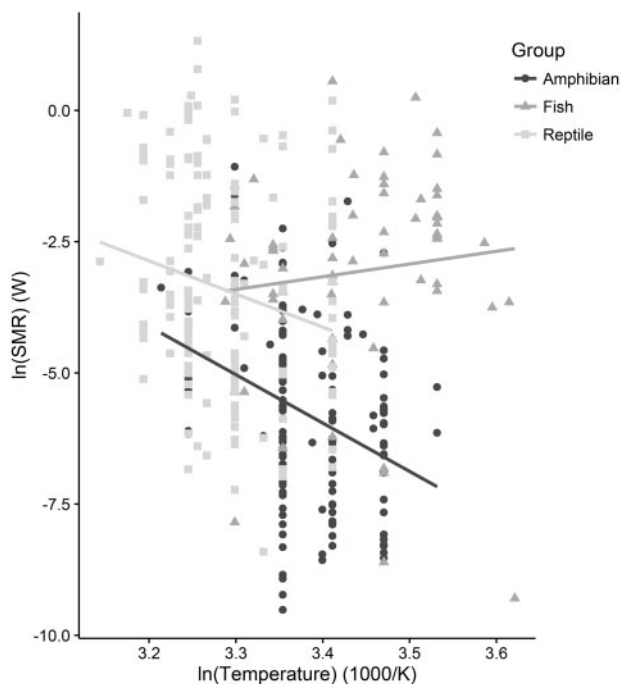
$$\text{Fish : } \ln B = -11.39 + 2.42(1000/T). \quad (2)$$

$$\text{Reptile : } \ln B = 17.34 - 6.31(1000/T). \quad (3)$$

$$\text{Amphibian : } \ln B = 25.37 - 9.21(1000/T). \quad (4)$$

For all taxonomic groups, temperature coefficients were significantly different from zero for reptiles ( $\beta = 6.31 \pm 2.49$ ;  $P = 0.012$ ) and amphibians ( $\beta = 9.21 \pm 2.16$ ;  $P < 0.001$ ), but not for fish ( $\beta = 2.42 \pm 2.77$ ;  $P = 0.385$ ; Fig. 2). Residuals from these regressions were extracted, back-transformed to their original scale, and used as temperature-corrected SMRs in subsequent analyses.

Finally, to confirm that our taxonomic groupings (following Gillooly et al. 2001; Nagy 2005; White et al. 2006; Downs et al. 2008) were appropriate for this level of analysis, we performed further analyses on subgroups within reptiles. Our compilation of metabolic rate data included 152 squamates (lizards and snakes), 7 turtles, and 4 crocodilians, a sample size that allows separate partitioning only for squamates. We thus used this group to determine whether the equations for lizards and snakes were similar to the general reptile equation described above (Equation 3). We found a significant difference between the temperature-corrected metabolic rates obtained from the analysis including all reptiles and the analyses including only data from snakes ( $t_{62} = -26.3$ ,  $P < 0.001$ ) or only data from lizards ( $t_{86} = 9.83$ ,  $P < 0.001$ ). The mean difference in temperature-corrected metabolic rate for these two modeling approaches was 1.98 W for lizards and



**Fig. 2** Standard metabolic rate (natural log-transformed; SMR) by temperature (natural log-transformed) by taxonomic group. Shaded areas denote 95% confidence intervals.

2.55 W for snakes. Given the large taxonomic scale of HormoneBase and the range of metabolic rates in our metabolic rate database (range: 0.0013–1377.3 W) the magnitude of differences in estimates from these two analyses are negligible, supporting the level of taxonomic partitioning used here.

### Taxonomic and geographic bias in hormone and life history measures

As HormoneBase aimed to include all published reports of plasma androgens and glucocorticoids that met our inclusion criteria (i.e., measures from adult, free-living animals that had not been experimentally manipulated, in which data from the sexes were reported separately), these data allow us to quantify whether taxonomic or geographic biases exist in the study of hormones in vertebrates. Using the data compiled and extracted as described above, we performed Chi-square tests of independence to determine the extent of taxonomic and geographic bias in the hormone and life history measures available in the primary literature.

To determine the total number of extant species in each major group of vertebrates, we used estimates from the OneZoom Tree of Life (onezoom.org; Rosindell and Harmon 2012), whose estimates are derived from the Open Tree of Life (<https://tree.opentreeoflife.org>). We compared the

**Table 1** Distribution of species across major taxonomic groups, number of species represented in HormoneBase, and percent of total species in HormoneBase

	Total number of species	Hormone Base species	% species included in Hormone Base
Crocodylians	23	3	13.04
Turtles	233	15	6.44
Birds	10,000	225	2.25
Mammals	5,043	59	1.17
Squamates	10,039	73	0.73
Amphibians	7445	40	0.54
Sharks and Rays	1255	6	0.48
Fishes	32,146	53	0.17
Total	66,184	474	0.72

total numbers of species in each group to the number of species represented in HormoneBase (Table 1). Overall, HormoneBase includes 0.72% of the total number of jawed vertebrates, and the representation of some species groups (particularly, crocodylians, turtles, and birds) is proportionally greater than others (particularly, fishes;  $\chi^2 = 639$ ,  $df = 7$ ,  $P < 0.001$ ). This bias may in part be due to the restrictions we placed on data for inclusion in HormoneBase, as we required circulating plasma measures of hormones, yet hormones in many aquatic organisms are collected from water samples. Indeed, the three most underrepresented taxa in HormoneBase (fishes, sharks and rays, and amphibians) are all aquatic. Yet it remains clear that fish, and to a lesser extent, amphibians, are undersampled in ecological endocrinology studies. In order to study hormones across the vertebrate tree of life in a robust way, we need more measurements of fish and other aquatic organisms under standardized conditions.

In addition, we examined the extent to which the locations from which hormone measures have been collected are biased with regard to terrestrial biomes. Using the mean precipitation and temperature data from 1965 to 2015 for each locality, we mapped the 456 terrestrial localities included in HormoneBase on a Whittaker plot (Fig. 3). We used the relative area each biome covered in the Whittaker plot as an estimate of the relative area of climate space encompassed by that biome (Table 2). This analysis did not include aquatic localities, or the nine localities that fell outside the range of the traditionally-defined biomes in the Whittaker plot (Fig. 3). Our results show a substantial bias toward adequate coverage of the

climate space encompassed by woodland shrubland and temperate forests, but a lack of coverage of climate space occupied by tropical and temperate rainforests ( $\chi^2 = 262$ ,  $df = 8$ ,  $P < 0.001$ ). This bias likely results from at least two sources: reduced overall hormone sampling in some biomes, and sample collection in some locations (e.g., tropical rainforest, tundra) being more likely to occur at specific field stations or long-term research sites, rather than being distributed across different localities within a given biome. Identifying this bias points researchers toward undersampled biomes for future endocrinological field studies.

Finally, considering only the species for which hormones have been measured (i.e., species represented in HormoneBase), we explored the extent of taxonomic bias in available life history traits (Table 3). For species in each of our five major taxonomic groups, we evaluated whether data were available for male and/or female

body mass, maximum longevity, number of reproductive attempts per year, and metabolic rate. We found no bias in the taxa for which body mass or longevity were reported, but significant bias in the taxa for which reproductive attempts per year and metabolic rate were reported (Table 3). In terms of reproductive attempts per year, amphibians and fish were underrepresented in the data that were available. This is likely due to a combination of the difficulty of obtaining such data in wild populations, and in some cases, challenges in defining what constitutes a single reproductive attempt in some mating systems (e.g., fish that spawn multiple times over several days or weeks). But, as this information is critical in determinations of reproductive value, these data are a critical gap in our understanding of these groups. In terms of metabolic rate, fish are again underrepresented, while mammals are somewhat overrepresented in the available data. The collection of metabolic rate data on a broader diversity of fish species thus reflects a valuable opportunity for future investigation.

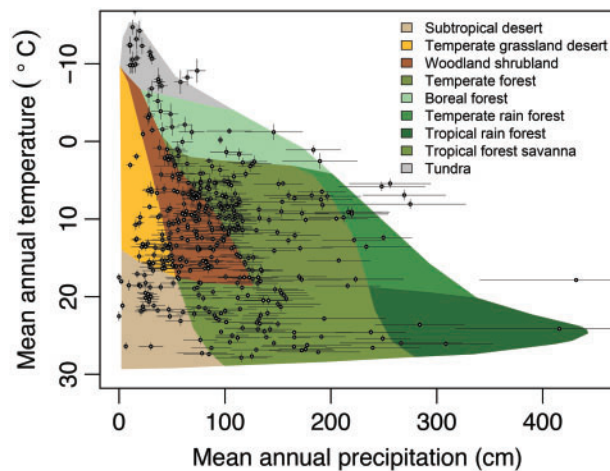


Fig. 3 Individual study locations plotted over Whittaker's terrestrial biome plot. Points represent annual mean temperature and precipitation for 1965–2015. Error bars denote standard deviation in annual temperature and precipitation.

Table 2 Distribution of localities in HormoneBase across biomes

	Number of localities in Hormone Base	% area in Whittaker plot	% localities in Hormone Base
Subtropical desert	37	10	8
Temperate grassland desert	39	7	9
Woodland shrubland	135	10	30
Temperate forest	125	20	27
Boreal forest	21	9	5
Temperate rain forest	7	8	2
Tropical rain forest	4	12	1
Tropical forest savanna	65	19	14
Tundra	23	4	5
Total	456		

Table 3 Distribution of available data for life history traits, across major taxonomic groups in HormoneBase

	Number of species in HormoneBase	Number of species body mass	Number of species longevity	Number of species clutch/year	Number of species metabolic rate
Amphibians	40 (8.4%)	34 (7.4%)	23 (5.5%)	22 (5.2%)	19 (9.5%)
Birds	225 (47.5%)	225 (48.7%)	213 (50.6%)	219 (51.9%)	97 (48.7%)
Fish	59 (12.4%)	53 (11.5%)	46 (10.9%)	32 (7.6%)	12 (6.0%)
Mammals	59 (12.4%)	59 (12.8%)	58 (13.8%)	59 (14.0%)	39 (19.6%)
Reptiles	91 (19.2%)	91 (19.7%)	81 (19.2%)	90 (21.3%)	32 (16.1%)
Total number of species	474	462	421	422	199
$\chi^2$		1.2	6.7	16.8	16.1
$P$		0.87	0.15	0.002	0.003



## Summary

In sum, here we provide the context and detailed methodology underlying HormoneBase analyses, in order to make this resource more useful to the scientific community. We also describe the decision-making approach used in the compilation of these data to allow this large-scale database to be used in subsequent statistical analyses. Further, our initial review of phylogenetic, environmental, and life history data associated with HormoneBase reveals substantial bias in the collection of circulating hormone measures in wild-living vertebrates, in terms of both taxonomy and geography. It is our hope that this information will allow the HormoneBase database (Vitousek et al. 2018) to be a broadly useful resource for animal biologists addressing a diversity of evolutionary questions.

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## Supplementary data

Supplementary data available at *ICB* online.

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