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Short Communication

Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species

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ABSTRACT

We developed and validated a non-invasive thyroid hormone measure in feces of a diverse array of birds and mammals. An I^{131} radiolabel ingestion study in domestic dogs coupled with High Pressure Liquid Chromatography (HPLC) analysis, showed that peak excretion in feces occurred at 24–48 h post-ingestion, with I^{131}-labelled thyroid hormone metabolites excreted primarily as triiodothyronine (T3) and relatively little thyroxine (T4), at all excretion times examined. The immunoreactive T3 profile across these same HPLC fractions closely corresponded with the I^{131} radioactive profile. By contrast, the T4 immunoreactive profile was disproportionately high, suggesting that T4 excretion included a high percentage of T4 stores. We optimized and validated T3 and T4 extraction and assay methods in feces of wild northern spotted owls, African elephants, howler monkeys, caribou, moose, wolf, maned wolf, killer whales and Steller sea lions. We explained 99% of the variance in high and low T3 concentrations derived from species-specific sample pools, after controlling for species and the various extraction methods tested. Fecal T3 reflected nutritional deficits in two male and three female howler monkeys held in captivity for translocation from a highly degraded habitat. Results suggest that thyroid hormone can be accurately and reliably measured in feces, providing important indices for environmental physiology across a diverse array of birds and mammals.

1. Introduction

Thyroid hormones (thyroxine, T4, and triiodothyronine, T3) have received little attention in environmental physiology compared to glucocorticoid and gonadal hormones, even though the former provide a great deal of complementary information. Thyroid hormones have profound influences on metabolism, heart rate, blood pressure, nutritional physiology, brain development and body temperature regulation independent of muscle activity (Oppenheimer, 1999; Silva 2003, 2006). Thyroid hormones are particularly responsive to nutritional deficits, lowering metabolism and allowing the body to conserve energy during a nutritional emergency (van der Heyden et al., 1986; Eales, 1988; Hennemann and et al., 1988; Blake et al., 1991; Flier et al., 2000; Douyon and Schteingart, 2002), but appear unaffected by psychological stress (Schew et al., 1996; Geris et al., 1999; Kitaysky et al., 2005, see also Walpita et al., 2007). By contrast, glucocorticoid (GC) measures increase in circulation (Sapolsky et al., 2000; Wingfield and Romero, 2001; Kitaysky et al., 2005) and feces (Wasser et al., 1997, 2000) in response to both psychological and nutritional stress. Combining thyroid and GC hormone measures thus helps characterize the role of reduced food availability in disturbance biology, enabling environmental disturbance impacts to be partitioned into nutritional and non-nutritional stress. Such information is critical for designing effective mitigation efforts and improving our understanding of allostatic load – a concept used to redefine the stress response in energetic terms (McEwen and Wingfield, 2003).

Thyroid hormone measures may also provide a valuable index of climate change impacts among free-ranging wildlife. Homeotherms adjust thyroid hormone to maintain a constant body temperature when outside their thermal neutral zone (Silva 2003,

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Table 1
Details of each species used in this study. All parallism and accuracy studies used sample pools from three individuals. VM = volume:mass study; F = female; M = male.

Common name	Scientific name	Location	Wild or captive	Time of collection	Collection and storage	Number of samples	Number of individuals[a]
Domestic beagles	*Canis domesticus*	University of WA lab animal	Captive, domestic	April, 2005	Fresh frozen	16 fecal, 43 urine	2F
Maned wolf	*Chrysocyon brachyurus*	Emas NP, Brazil	Wild	August, 2007	<2 Week, stored frozen	48 VM	3
Gray wolf	*Canis lupus*	NE Alberta	Wild	January, 2007	Fresh frozen	32 VM	2
Woodland caribou	*Rangifer tarandus*	NE Alberta	Wild	January, 2007	Fresh frozen	32 VM	2
Moose	*Alces alces*	NE Alberta	Wild	January, 2007	Fresh frozen	32 VM	2
African elephant	*Loxodonta africana*	Mikumi NP, Tanzania	Wild	July, 2006 and 2008	<2 Week, rapid air dry	8 VM	1
Howler monkey	*Alouatta palliata*	Veracruz, Mexico	Wild	June, 2007	Fresh frozen	163 nutrition	2 M, 3F
Killer whale	*Orcinus orca*	Puget Sound, WA	Wild	August, 2007 and 2008	Fresh frozen	46 VM	3
Steller sea lion	*Eumetopias jubatus*	Vancouver Aquarium	Captive	June, 2007	Fresh frozen		3
Northern spotted owl	*Strix occidentalis caurina*	Shasta NF, CA	Wild	May, 2008	Fresh frozen	24 VM	1 F

[a] This is maximum number, assuming each wild sample was from a unique individual.

2006). Thyroid hormones decrease when temperatures rise above the species' thermal neutral zone and increase when temperatures fall below it (Silva 2003, 2006).

The excretion of thyroid hormone in bile of birds and mammals (Taurog et al., 1951; DiStefano, 1988; DiStefano et al., 1987) creates an opportunity to measure thyroid hormones in feces, providing a highly accessible, non-invasive tool with enormous potential for understanding environmental physiology at individual and population levels. To this end, we developed and validated measures of thyroid hormones excreted in feces of a variety of avian and mammalian species.

We assessed the excretion metabolites of thyroid hormone by administering radiolabeled I^{131} in capsule form to two domestic dogs. Thyroid hormone is the only major biochemical molecule known to incorporate iodine. Thus, ingested I^{131} is rapidly converted exclusively to thyroid hormone, allowing us to track the entire metabolic process from synthesis through excretion of thyroid hormone, including its metabolic forms. The same method is routinely used to evaluate thyroid function in clinical medicine. We next optimized and validated extraction and assay methods for the thyroid hormone fecal metabolites in one bird and eight mammal species, all but one of which was free-ranging (Table 1). These measures were then used to characterize the thyroid hormone response to changes in feeding behavior of two male and three female Mexican mantled howler monkeys (*Alouatta palliata*) during their translocation from a highly degraded habitat.

2. Methods

2.1. Study species

The two domestic female beagle dogs, Nyoko and Yunko, used in the radiolabel infusion study were housed in separate 6′ × 8′ indoor fenced runs, with sloping, painted concrete floors and a center drain. Both dogs weighed approximately 3 kg. Nyoko was notably much more energetic than Yunko. The dogs were fed a standard canine dry food diet. All work was approved by the UW IACUC protocol 2850–05.

Details of the owl and eight mammalian species (two carnivores, three ungulates, and one primate, cetacean and pinniped) used in the fecal sample validation studies are described in Table 1. All species were free-ranging except for the Steller sea lion. The two male and three female howler monkeys in the feeding study were captured for translocation on April 24th 2007 from a highly degraded forest fragment in a 1600 m^2 area at the edge of a cattle ranch in the Cascajal del Río, municipality of Acayucan, Veracruz, Mexico. Prior to capture, they fed primarily on leaves of two species (*Tabebyia pentaphylla* and *Andira inermis*). Fecal samples were collected *ad libitum* for 1 week immediately pre-capture, and continuously for their first 2 weeks post-capture. The monkeys were housed outdoors, in separate contiguous fenced cages with concrete floors at the Institute of Neuroethology, in Catemaco, Veracruz, and provided an *ad libitum* diet of fresh fruit and daily cut branches with young leaves (*Ficus tecolutensis*, *Bursera simaruba*, *Vitis lilifolia, Hidalgoa ternate, Shingonium phodophilum*) before being translocated. However, the females ate relatively little during their first two weeks in captivity. Animal care was in accordance with local, state and federal regulations for animal welfare in Mexico.

2.2. I^{131} Radiolabel ingestion study

Three μCi of NaI^{131} were fed in capsule form to each 3 kg beagle dog. The I^{131} is sequestered by the thyroid gland and used to synthesize primarily thyroxin (T4), and some triiodothyronine (T3) and a very small amount of reverse T3 (Tomasi, 1991). All urine and feces were collected hourly from both dogs for 96 h post-ingestion to quantify excretion of T4, T3 and rT3 as well as any other metabolites bound to I^{131}. Urine was collected in a plastic cup inserted into the drain, whereas, feces were collected directly off the floor. The time of each collection was recorded and the floor was wiped down with a clean ethanol-saturated cloth after each collection. Both dogs were adopted into homes following the procedure.

All urine and feces were thoroughly homogenized, and three sub-samples of each (1 ml urine, 0.6 g wet feces) immediately counted (2 min) for radioactivity on a Perkin–Elmer Wizard 1470–020 gamma counter on the day of collection.

Fecal extracts from the peak and secondary peak radioactive fecal samples (samples, respectively collected at 21 h (peak) and 53 h for Nyoko, and 45 h (peak) and 98 h for Yunko) were prepared for HPLC analysis of excreted metabolites using the method derived for thyroid hormone separation described in Kobuke et al. (1987), with some modification for fecal samples based on Wasser et al. (1994, 2000). Briefly, ~1.2 g of each fecal sub-sample was weighed into a 16 × 100 mm borosilicate glass tube, extracted in 4.00 ml of 95% ethanol (5% distilled water; Kobuke et al., 1987) using a multitube, pulsing vortexer (30 min at 1 pulse/s) and centrifuged (15 min at 2200 rpm). The extract was then passed through a 2 μm filter, evaporated and reconstituted in citrate buffer (pH 4.0), loaded onto a C-18 Bond Elut Jr. cartridge, and eluted in 100% methanol. The eluent was further concentrated and 100 μl injected onto a C-18 reverse phase HPLC column (Wasser et al., 2000, 1994). HPLC analysis used a methanol/0.02 M ammonium

acetate (pH 4.0) gradient, with methanol increasing linearly (47–65% over 40 min) to separate T4, T3, rT3, T2s, and T1s (Sweeting and Eales, 1992). Each 1 min fraction collected over 40 min was counted for radioactivity and compared to elution profiles of pure T4, T3, and rT3.

HPLC analysis of fecal and urine metabolites were conducted within 3 days of sample collection owing to the 8 day half-life of I^{131}. All HPLC fractions were then held for an additional 80 days to allow complete radioactive decay before assayed for immunoreactive T3 and T4.

2.3. Thyroid hormone radioimmunoassay methods

We compared the following I^{125} coated tube assays kits: Diagnostic Systems Laboratories (DSL, Webster, TX) Total T3 (DSL-3100), MP Biomedicals (Orangeburg, NY) Free T3 (06B258709) and Total T3 (06B254215) and Diagnostic Products Corporation/Siemens (Los Angeles, CA) Total T4 kit (catalog #TKT45).

In all cases, assays were performed according to kit instructions with the following modifications: Standards were made from crystalline T3 and T4, respectively, dissolved in ethanol and diluted in phospho-saline-BSA buffer (pH 7.4: 0.25 g BSA, 2.71 g NaH_2PO_4, 4.6 g Na_2HPO_4, 50 ml 0.5 M EDTA, pH 7.5, 950 ml distilled H_20 per litre). A volume of the standard, ranging from 125 to 750 μL, depending on the species' optimal dilution, was dried under forced air in a 12 × 75 mm borosilicate glass tube and resuspended in 250 uL assay buffer.

DSL discontinued their kit during the course of our study so all subsequent assays used the MP Biomedicals total T3 kit (06254282). Fortunately, the overall patterns between the DSL and MP total kits were virtually identical both longitudinally and across HPLC fractions (see Results).

The cross-reactivities of the MP total T3 antibody to other compounds was: L-triiodothyronine 100%, L-thyroxine 0.18%, 3,3′5′-L-triiodothyronine (r-T3) 0.01%, 3,5-diiodothyronine 0.44%, and <0.01% for 3,5-diiodotyrosine, phenylbutazone, sodium salicylate, diphenylhydanation and dicumerol.

The specific cross reactivity of the T4 antibody to other compounds was: L-thyroxine 100%, D-thyroxin 64%, tetraidothyroacetic acid 104%, triiodo-L-thyronine 2%, triidothyroacetic acid 2%, and nondetectable levels of triiodo-D-thyronine, monoidotyrosine, diiodo-L-tyrosine, methimazole, 5,5′ diphenylhydantoin, phenuylbutazone, and 6-*n*-propyl-2-thiouracil.

2.4. Extraction optimization and validation

Initial studies compared extraction efficiency using methanol versus ethanol at concentrations of 50%, 60%, 70%, 80% and 90%. The highest recoveries occurred using 70% ethanol, leading to the final extraction method: Homogenized samples are freeze-dried prior to extraction, allowing hormone concentrations to be expressed per gm dry weight, while controlling for intersample variation due to diet and variable moisture (Wasser et al., 1993). Fifteen milliliter of 70% ethanol was added to 0.1 g of freeze-dried and thoroughly homogenized fecal powder, vortexed on a multitube pulsing vortexer for 30 min at 1 pulse/s (Glas-Col, Terre Haute, IN), and then centrifuged for 20 min at 2200 rpm. The supernatant was decanted from the fecal pellet and stored in an airtight tube. The original pellet was re-extracted (15 ml, 70% ethanol) a second time, extracts combined and stored at −20 °C until assayed.

2.5. Extraction recovery, parallelism and accuracy studies

The following method aimed to test detectability of high versus low T3 concentrations across species, as well as derive the ratio of volume of ethanol to sample mass that yielded the highest extraction efficiency. We focused on T3 because this was the major excretion product in the I^{131} ingestion study (see below). Multiple high and low concentration sample pools were generated from two or more individuals of each wildlife species, except for the northern spotted owl (owing to small fecal size) and African elephants (high and low samples were no longer available at the time of these experiments; thyroid hormone degraded in feces after one year in storage).

Each species-specific sample pool was extracted in paired sample masses of 0.1 and 0.2 g of freeze-dried feces, and extracted separately in 5, 10, 15 and 20 ml of 70% ethanol. The remainder of the extraction process was identical to the final method detailed above. The owl sample pool consisted of a single well-homogenized

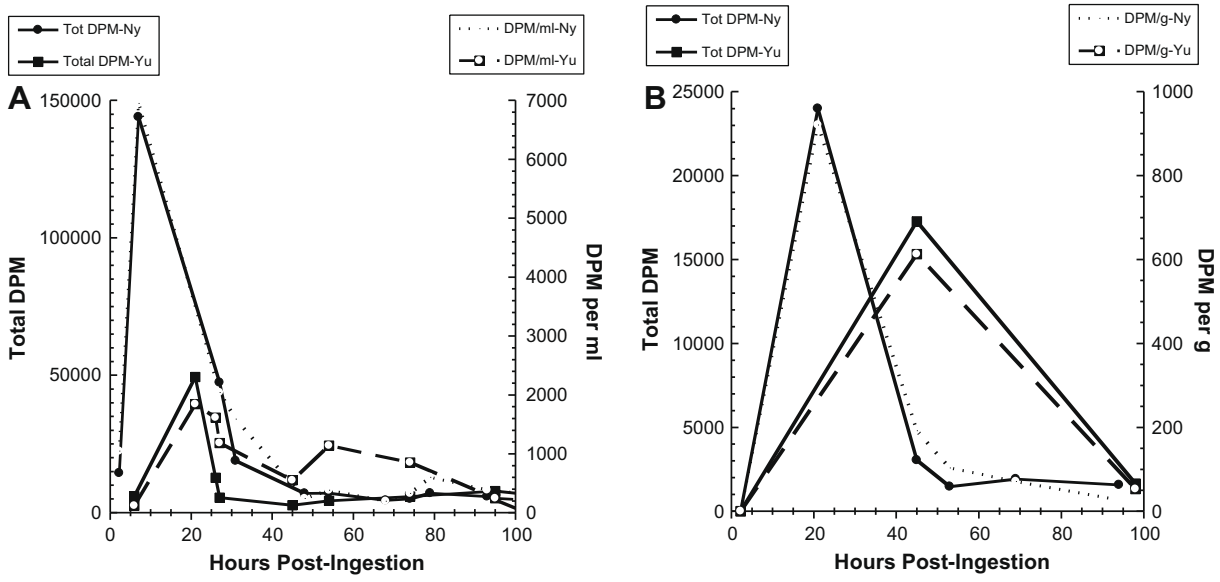

Fig. 1. Excretion profiles of I^{131} in (A) urine and (B) feces of two domestic dogs, Nyoko and Yunko, following ingestion of 3 μCi I^{131}. Figures show total DPM excreted by Nyuko (solid circle and line) and Yunko (solid square and line), and DPM per ml urine and per gram feces excreted by Nyuko (open circle, dotted line) and by Yunko (open square, dashed line).

pool of fecal samples, divided into groups of 0.025, 0.05 and 0.1 to be more representative of sample sizes collected in the field and extracted in 2–15 ml ethanol. Final T3 concentrations were then compared across all volume:mass groups within and between species (see Statistical Analyses).

Recovery of radiolabeled T3 and T4 was also tested using the optimized assay of 0.1 g feces in 15 ml 70% ethanol (and 0.025 g feces in 15 ml for the owls). About 50,000 cpm of I^{125}-T3 or I^{125}-T4 were added to total count tubes and to respective separate samples from each species in duplicate. Post-extraction recovery counts were then compared to the respective total counts for each hormone.

Serially diluted sample pools were also used to examine the T3 and T4 assays for parallelism and accuracy using the final (most efficient) extraction method. All hormones were measured as nanograms hormone per gram dry feces (ng/g) and sample extracts outside 15–85% bound on the standard curve or with a coefficient of variation between duplicate pairs $\geqslant$10% were re-assayed at appropriate dilutions.

2.6. Statistical analyses

Assay parallelism was examined using an F ratio test in the software PRISM to test for differences in slopes. A general linear model was used to predict T3 concentration of pooled high and low concentration samples for each species, after controlling for species and the ratio of ethanol volume to sample mass (in linear and quadratic forms to test for an asymptote in T3 recovery with increasing ethanol volume). The interactions between species and ethanol volume/mass, and species and high or low concentration were also examined. T3 was log-transformed for normality in this and all other analyses. A general linear model was also used to test for change in T3 over time, as well as the interaction between sex

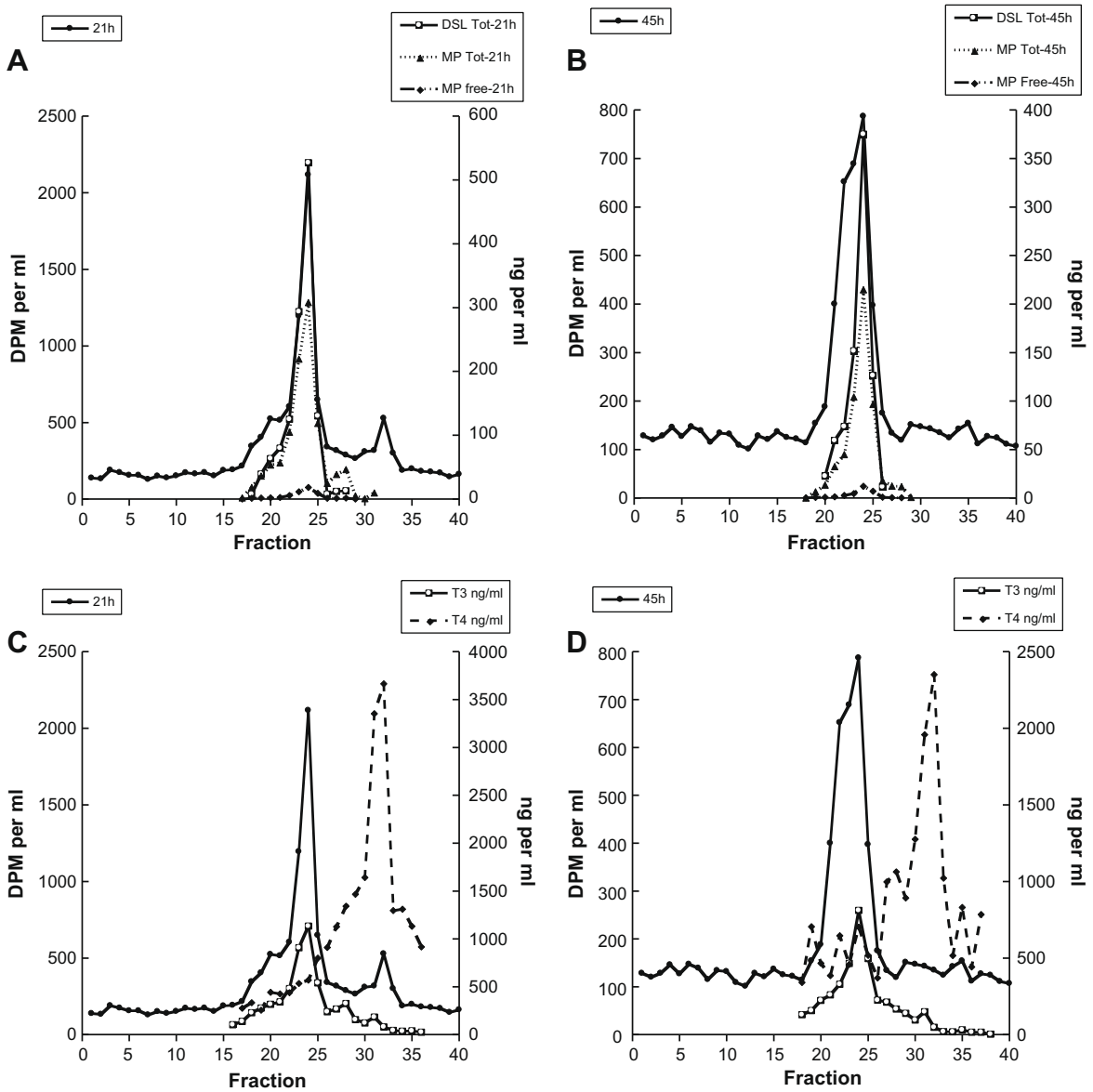

Fig. 2. Elution profiles of I^{131}-labelled fecal metabolites and their associated immunoreactivies for T3 (A and B) and T4 (C and D) following HPLC separation of the peak radioactive samples (shown in Fig. 1B), 21 h after ingestion of 3 μCi I^{-131} in Nyoko (A and C) and 45 h after ingestion in Yunko (B and D). DPMs for I^{131}–labelled fecal metabolites shown by solid circle and line in A–D. T3 immunoreactivities are shown for the DSL total T3 antibody (open square, solid line), MP total T3 antibody (solid triangle, dotted line), MP free T3 antibody (solid diamond, dashed line) in A and B. T4 immunoreactivity using the DPC total T4 antibody (solid diamond, dashed line) is shown relative to the MP total T3 antibody (open square, solid line) in C and D. Pure T3 elutes in fractions 23–24; T4 elutes in fraction 32; and rT3 elutes in fraction 29.

and time, in the howler monkey samples collected prior to, and for the 2 weeks after capture.

3. Results

3.1. Ingestion of I^{131} in two domestic dogs

Excretion profiles from time of I^{131} ingestion in the two domestic dogs varied between individuals for both urine and feces. Urine excretion of I^{131} in the more energetic dog (Nyoko) was first detected at 2.25 h post-ingestion, with a peak at 6.5 h, compared to the less energetic dog (Yunko) with first urine excretion at 6.5 h and a peak at 21 h (Fig. 1A). Nyoko also excreted two times the total amount of I^{131} as Yunko in her urine. In feces, the first detected excretion was also the peak, occurring at 21 h in Nyoko and 45 h in Yunko, with Nyoko excreting 1.5 times more radioactivity than Yunko (Fig. 1B).

Both dogs excreted similar ratios of I131-labeled T3:T4 at all sample collection times (Fig. 2A and B). Nearly all radioactivity in peak samples excreted at 21 and 45 h post-ingestion by Nyuko and Yunko, respectively, eluted in fractions 23–24, which are the same fractions where pure T3 elutes. A relatively small amount of radioactivity also eluted in fraction 32, where pure T4 elutes. Very little, if any, radiolabeled rT3 was found in feces (fraction 29). The same patterns were found in all fecal samples collected, regardless of excretion time, as well as in urine (data not shown).

We compared the immunoreactivity detected by free and total T3 antibodies from MP Biomedicals (Orangeburg, NY) and the total T3 antibody from DSL (Webster, TX) across these same HPLC fractions (Fig. 2A and B). All three T3 assays showed nearly perfect correspondence in elution time with the shed radioactive T3 metabolites ($r = 0.995$ for DSL total T3; $r = 0.968$ for MP total; $r = 0.96$ for MP free). The immunoreactive profiles of the three different T3 assays were virtually identical but markedly higher concentrations were obtained from the two total versus the free antibody kits, with highest overall concentrations from the DSL total T3 antibody kit. Since the DSL kit was discontinued, the remainder of T3 assays in this paper used the MP total T3 kit.

Fig 2C and D shows immunoreactivity of the DPC T4 kit to the same HPLC fractions. Immunoreactivity of the MP total T3 antibody is also plotted for comparison. Far more immunoreactive T4 was excreted relative to immunoreactive T3, even though I^{131} was disproportionately excreted as T3 in both dogs. T3, the more bioactive of the thyroid hormones, thus appears to be excreted in direct proportion to its synthesis. However, more immunoreactive T4 is excreted overall, in concert with its relatively high availability in circulation (Norman and Litwack, 1997). (The immunoreactivity of rT3 was not tested.)

3.2. Recovery, parallelism and accuracy studies

Recovery of I^{125}-labeled T3 and T4 added to fecal samples immediately prior to extraction varied across species from 67% to 99% for T3 and 40% to 100% for T4 (Table 2), with T3 and T4 recoveries consistently highest in the herbivorous mammals.

Parallelism and accuracy studies of T3 and T4, using the MP Biomedicals total T3 antibody and the DPC T4 antibody, respectively, are shown by species in Table 2. Contrary to the recovery experiments, T4 was undetectable in the herbivorous mammals (Table 2). Serial dilutions paralleled respective standard curves for T3 and T4 (where detectable) in all cases and fifty percent binding occurred at a 1:10–1:30 dilution, depending on species (Table 2). Accuracy studies produced slopes ranging from 0.95 to 1.1, illustrating that fecal extracts were not interfering with measurement precision (Table 2). Thus, these assay accurately and reliably measured T3 and T4, respectively, across their species-specific ranges of concentration, without interference of fecal products. Inter-assay cv's ranged from 1.2% to 3.0% for T3 and T4. Intra-assay cv's ranged from 3% to 10% (Table 2).

Table 2
Dilutions at 50% binding from parallelism studies, slopes from accuracy studies, cv's and extraction recoveries for (A) T3 and (B) T4, across diverse avian and mammalian species. T3 assay: MP Biomedicals Total T3 RIA kit; T4 assay: DPCs T4 RIA kit. All *F*-tests comparing slopes of standard curves with those of serial dilutions were non-significant, indicating good assay parallelism for all species. All analyses for slope in accuracy studies had an $r^2 \geqslant 0.96$. NSO = northern spotted owl; SSL = Steller sea lion.

Species	Dilution	Slope	Inter-assay CV	Intra-assay CV	% Recovery
(A)					
Afr. elephant	1:10	1.08	1.9	7.8	96
Moose	1:10	1.06	1.8	2.9	96
NSO	1:10	1.09	1.2	9.9	
Caribou	1:20	0.99	2.1	9.4	83
Maned wolf	1:20	0.99	2.6	9.0	99
Orca	1:30	1.09	1.9	9.9	68
Wolf	1:30	1.08	1.8	9.4	79
SSL	1:60	0.94	1.3	9.8	84
Howler	1:60	1.13	3.0	5.1	
(B)					
Afr. elephant		Undetectable			100
Moose		Undetectable			100
NSO	1:5	1.05	2.2		
Caribou		Undetectable			100
Maned wolf	1:5	1.0	1.8	7.9	99
Orca	1:15	1.09	2.2		42
Wolf	1:30	1.05	2.9		32
SSL	1:30	0.94	1.8		40
Howler					Undetectable

Table 3
Species-specific results of statistical analyses distinguishing high and low T3 concentration samples, after controlling for the ethanol volume:sample mass ratio. All $r^2 \geqslant 0.96$ for mammals, $r^2 = 0.68$ for spotted owl; all $p < 0.0001$.

Species	Hi vs. Lo t, p	Volume/mass t, p	$(\text{Volume/mass})^2$ t, p	*N*
Afr. elephant	Not tested	19.8, 0.0001	−4.5, 0.0002	27
Caribou	−35.8, 0.0001	ns	ns	32
Moose	−47.5, 0.0001	1.83, 0.08	−1.8, 0.08	31
Wolf	−85.1, 0.0001	4.97, 0.0001	−2.19, 0.04	32
Maned wolf	−28.0, 0.0001	ns	ns	31
Killer whale	−31.6, 0.0001	7.6, 0.0001	−1.91, 0.07	31
Northern spotted owl	Not tested	7.6, 0.0001	−4.2, 0.0002	32

3.3. Extraction volume/sample mass impacts on T3 recovery

T3 concentration per sample pool was significantly predicted (overall $r^2 = 0.99$, $F = 995.6$, df = 13,168 $p = 0.0001$) by species ($F = 1140$, $p < 0.0001$), high versus low concentration ($F = 8990$, $p = 0.0001$), the ratio of ethanol volume to extracted sample mass ratio in linear ($F = 44.47$, $p < 0.001$) and quadratic forms ($F = 5.75$, $p < 0.02$), and the interactions of species with high or low concentration ($F = 235.46$, $p < 0.0001$) and with the volume/mass ratio ($F = 8$, $p < 0.0001$). T3 concentration, and hence recovery, was consistently highest for all species when using 0.1 g sample mass extracted in 15–20 ml of 70% ethanol, with a plateau in extraction efficiency at 15–20 ml. This analysis predicted nearly 100% of the variance in hormone concentrations, distinguishing high and low concentration samples across all five species tested (Table 3).

3.4. Howler monkey feeding study

After controlling for individual, fecal T3 levels showed a significant decline with time post-capture, independent of sex ($t = -3.2$,

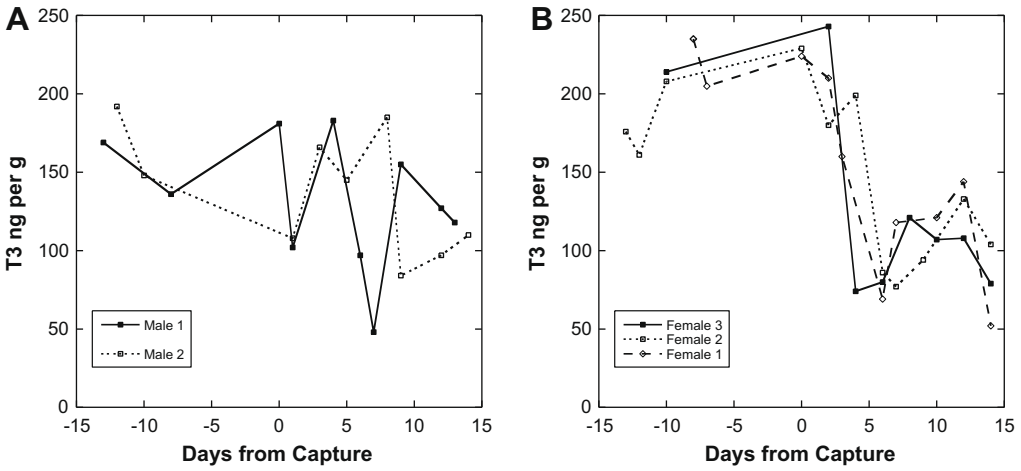

Fig. 3. T3 concentrations in (A) two male and (B) three female howler monkeys plotted as a function of days from capture (-days = pre-capture).

$p = 0.003$) (Fig. 3A and B) in the howler monkeys. However, dummy coding these variable to partition variance by each sex revealed the decline to only be significant for females (female: $t = -3.3$, $p = 0.003$; male: $t = -1.04$, $p = 0.3$; $r^2 = 0.29$, $F = 2.15$, df, 6,37, $p = 0.07$). These differences correspond to sex-differences in feeding since females fed relatively little compared to males post-capture.

4. Discussion

The thyroid gland is unique because it both secretes and stores thyroid hormones. The concentration of T4 in blood is 40–100 times that of T3. T4 secretion by the thyroid gland is 10 times that of T3, although 80% of secreted T4 is deionated in peripheral tissues to form T3 (Norman and Litwack, 1997). T3 is the bioactive thyroid hormone, being roughly eight times more potent than T4 (Tomasi, 1991). Our I^{131} ingestion study suggests that T3, the more potent form of thyroid hormone, is excreted in feces in direct proportion to its use, with peak excretion in feces 24–48 h post secretion. By contrast, T4, the less potent, storage form of thyroid hormone, is excreted more in proportion to its accumulated availability in circulation. Presumably, preferential excretion of T3 avoids sustained physiologic effects, with the body making more of this more potent hormone as needed, as is typical of many important hormones regulated by negative feedback. These excretion patterns may also explain the greater I^{131}-labeled hormone excretion in Nyuko compared to Yunko (Fig. 1). A greater portion of I^{131}-T4 may have been converted to I^{131}-T3 in Nyuko, the more active dog, leading to more rapid excretion of I^{131}-T3 in her feces. The majority of radiolabeled T3 is also cleared within 24 h in humans whereas up to half of radiolabeled T4 may be retained for more than one week (Chopra 1976).

The combined results of the I^{131} excretion study, extraction and assay optimization studies, and the T3 response to diminished food intake in the howler monkeys, suggest that thyroid hormone metabolites can be accurately measured in feces, providing a reliable vehicle for monitoring biological activity in thyroid hormone across a wide variety of birds and mammals. T3 (and T4) is best extracted in 70% ethanol, using a sufficient volume of ethanol (15 ml) relative to sample mass (0.1 g dry feces) to assure that the ethanol does not become saturated during the extraction process. A TSH challenge in four captive female Steller sea lions housed at the Vancouver aquarium by Keech et al. (2010), using methods validated in the present paper, also demonstrated biological activity of fecal T3, and less so T4. Serum T3 was also more responsive than T4 in a TSH challenge in humans (Beer et al. 1989).

Although both T3 and T4 are measurable in feces, T3 appears to be the more informative hormone to measure owing to its greater potency and an excretion profile more in proportion to it utilization, compared to T4. Given the many important physiological processes reflected by thyroid hormone (Oppenheimer, 1999; Silva 2006), fecal thyroid hormone measures should provide a valuable tool in environmental physiology, both by itself and as a complement to currently employed measures such as fecal glucocorticoids.

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