General and Comparative Endocrinology 226 (2016) 5–13

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Urinary iodine and stable isotope analysis to examine habitat influences on thyroid hormones among coastal dwelling American alligators

Ashley S.P. Boggs [a,b,*], Heather J. Hamlin [c], James C. Nifong [d], Brittany L. Kassim [a,b], Russell H. Lowers [e], Thomas M. Galligan [b,f], Stephen E. Long [a,b], Louis J. Guillette Jr. [b,f]

[a] National Institute of Standards and Technology, Environmental Chemical Sciences, 331 Fort Johnson Rd, Charleston, SC 29412, USA
[b] Hollings Marine Laboratory, 331 Fort Johnson Rd, Charleston, SC 29412, USA
[c] University of Maine, School of Marine Sciences, 316 Murray Hall Orono, ME 04469, USA
[d] University of Florida, Fisheries and Aquatic Sciences, NW 71st Street, Gainsville, FL 32653, USA
[e] National Aeronautics and Space Administration, InoMedic Health Applications Inc., SR 405, Kennedy Space Center, FL 32899, USA
[f] Medical University of South Carolina, Department of Obstetrics and Gynecology, 331 Fort Johnson Rd, Charleston, SC 29412, USA

ARTICLE INFO

Article history:
Received 8 June 2015
Revised 20 October 2015
Accepted 8 December 2015
Available online 9 December 2015

Keywords:
American alligator
Thyroid hormone
Thyroxine
Triiodothyronine
Urinary iodine
Stable isotope analysis

ABSTRACT

The American alligator, generally a freshwater species, is known to forage in marine environments despite the lack of a salt secreting gland found in other crocodylids. Estuarine and marine foraging could lead to increased dietary uptake of iodine, a nutrient necessary for the production of thyroid hormones. To explore the influence of dietary iodine on thyroid hormone health of coastal dwelling alligators, we described the seasonal plasma thyroxine and triiodothyronine concentrations measured by radioimmunoassay and urinary iodine (UI) concentrations measured by inductively coupled plasma mass spectrometry. We also analyzed long-term dietary patterns through stable isotope analysis of scute tissue. Snout-to-vent length (SVL) was a significant factor among UI and stable isotope analyses. Large adult males greater than 135 cm SVL had the highest UI concentrations but did not display seasonality of thyroid hormones. Alligators under 135 SVL exhibited seasonality in thyroid hormones and a positive relationship between UI and triiodothyronine concentrations. Isotopic signatures provided supporting evidence that large males predominantly feed on marine/estuarine prey whereas females showed reliance on freshwater/terrestrial prey supplemented by marine/estuarine prey. UI measurement provided immediate information that correlated to thyroid hormone concentrations whereas stable isotope analysis described long-term dietary patterns. Both techniques demonstrate that adult alligators in coastal environments are utilizing estuarine/marine habitats, which could alter thyroid hormone physiology.
Published by Elsevier Inc.

1. Introduction

Alligatorids including American alligators (*Alligator mississippiensis*) are distinct from closely related crocodylids in that they do not have lingual salt secreting glands, thereby limiting their use of marine and estuarine habitats (Taplin et al., 1982). However, alligators will forage in marine and estuarine habitats, provided there is a reliable nearby source of low salinity water (Nifong et al., 2015, 2014; Rosenblatt and Heithaus, 2011; Rosenblatt et al., 2013). The iodine content of estuaries has been shown to be dependent on the marine end member sources, with more iodine in the saltier environments (Cook et al., 2000; Truesdale and Upstill-Goddard, 2003). Additionally, marine prey contain more iodine than freshwater prey (Eckhoff and Maage, 1997). Therefore, coastal environments provide an iodine-rich diet compared to freshwater environments. Iodine is a dietary nutrient required for the production of the thyroid hormones thyroxine and triiodothyronine (T_4 and T_3, respectively), which contribute to regulation of growth, metabolism, and reproduction (Flamant and Samarut, 2003; Trokoudes et al., 2006; Umpleby and Russell-Jones, 1996). A diet overly rich in iodine can induce hypothyroidism or hyperthyroidism in some species (Markou et al., 2001; Martin et al., 2000; Stanbury et al., 1998) and has been linked to hyperthyroid biomarkers in neonatal American alligators (Boggs et al., 2013). It thereby follows that because iodine is elevated in saltwater and marine prey compared to freshwater prey items and iodine is a nutrient required for production of thyroid hormones, alligators in coastal regions could have altered thyroid hor-

* Corresponding author at: National Institute of Standards and Technology, Environmental Chemical Sciences, 331 Fort Johnson Rd, Charleston, SC 29412, USA.
E-mail address: ashley.boggs@nist.gov (A.S.P. Boggs).

http://dx.doi.org/10.1016/j.ygcen.2015.12.006
0016-6480/Published by Elsevier Inc.

mone concentrations due to an increased exposure to environmental iodine when foraging on marine/estuarine prey.

Merritt Island National Wildlife Refuge (MINWR) in Florida is located on a barrier island and houses the facilities for the National Aeronautics and Space Administration's (NASA) Kennedy Space Center (KSC). Although MINWR consists primarily of estuarine and marine ecosystems, it supports a robust alligator population. Activities at MINWR such as impoundment regulation for mosquito control and bird migrations, and retention ponds for KSC activities have created a variety of freshwater resources that potentially contribute to use of high salinity environments by the resident alligator population. Moreover, previous research at MINWR demonstrates alligators travel to and forage within associated marine and estuarine environments (Nifong et al., 2014). However, intra-population variation in these cross-ecosystem foraging behaviors has yet to be determined and can potentially have significant impacts on alligator health due to the effects of increased iodine on thyroid hormone synthesis.

Previous research has shown that neonatal alligators from saline habitats at MINWR exhibit a strong correlation between plasma inorganic iodide and the biologically active thyroid hormone, T_3, which was not observed in the two freshwater populations from the same study (Boggs et al., 2013). This relationship is not present in juvenile alligators from MINWR, as there was no correlation between plasma inorganic iodide and thyroid hormones despite a strong correlations between plasma inorganic iodide and T_3 among the neonatal population (Boggs et al., 2011). However, plasma inorganic iodine is a marker of circulating iodine, not iodine intake. It is possible that the larger/older alligators at MINWR may be regulating plasma iodide by increasing urinary iodine excretion, but this has not been explored previously.

Therefore, in this study, we describe the thyroid hormone seasonal cycles of sexually mature adult alligators at MINWR and examine urinary iodine (UI) concentrations to explore if excretion of excess iodine is correlated to thyroid hormone concentrations among different size classes and sexes of adult alligators inhabiting MINWR. We then use stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) to estimate the proportional contributions of marine/estuarine and freshwater/terrestrial prey to the diet of juvenile and adult alligators. Combined, these analyses provide a detailed assessment of intra-population variation in marine foraging and the potential implications of these behaviors for alligator endocrinology and health.

2. Materials and methods

2.1. Animals and tissue/fluid sampling

All animal care and use was performed in accordance with the University of Florida Institutional Animal Care and Use Committee (IACUC) under Protocol No. 201005071 and IACUC GRD-06-044. Samples were collected under MINWR permit 2006 SUP 55 and permits from the Florida Fish and Wildlife Conservation Commission and U.S. Fish and Wildlife Service.

Blood sampling and morphometrics were collected according to Myburgh et al. (2014) and specifics for this collection can be found in Hamlin et al. (2011). Briefly, all animals (2006–2010; $n = 595$) were actively captured at MINWR and KSC and an immediate blood sample taken upon restraint. Blood was collected in a lithium heparin Vacutainer (BD 367884), kept on ice until returned to the laboratory, and centrifuged to obtain plasma. Aliquots were frozen at −20 °C.

Urine samples ($n = 174$) were obtained from 2007 to 2010 using the method described by Myburgh et al. (2012). Briefly, urine was collected in 50 mL Falcon tubes using a canine urinary catheter inserted into the urodeum via the cloaca. Urine was kept on ice until returned to the laboratory and then frozen at −20 °C.

From 2006 to 2013 a single caudal scute (superficial scale-like structures of the integument; $n = 314$) was removed from captured individuals using a sterile scalpel, and used for stable isotope analysis. Scute samples were placed on ice in the field and immediately frozen at −20 °C in the laboratory until further processing. After cleaning with deionized water to remove foreign debris, the keratinous epidermal layer was separated from dermal collagen layer of the scute using NaOH digestion (Alibardi and Thompson, 2000; Radloff et al., 2012). Isolated keratin samples were dried for 48 h at 60 °C and ground to fine powder. *A. mississippiensis* scute tissue has a slow rate of turnover (mean turnover = 590 days for $\delta^{13}C$ and 414 days for $\delta^{15}N$) (Rosenblatt and Heithaus, 2013) thus stable isotope signatures of scute keratin represent incorporation of long-term dietary patterns (>1 year).

In 2013, samples of representative alligator prey species from both marine/estuarine and freshwater habitats within MINWR were collected to calculate end member values for use in our isotopic mixing model analysis. Prey item isotope samples were collected by the Florida Fish and Wildlife Conservation Commission – Fish and Wildlife Research Institute's Fisheries-Independent Monitoring Program. Collection and processing of tissue samples followed methods described in Nifong et al. (2015).

2.2. UI measurements

Two bottles each of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 3668 Frozen Human Urine, Level 1 and Level 2 having a certified value of 142.7 μg/L ± 1.6 μg/L and 279.0 μg/L ± 3.9 μg/L, respectively, were used as control materials for iodine measurements.

Urine samples were agitated before approximately 0.5 mL was pipetted into a 15 mL polypropylene centrifuge tube followed by the addition of 5 mmol/L sodium hydroxide volumetrically diluting up to 10 mL. Samples resulting in high iodine concentrations, beyond the calibration curve, were further diluted by half and analyzed. In addition to the samples and control materials, twenty-one process blanks were carried through the entire sample processing and measurement scheme. The average blank mass fraction of iodine was 0.12 μg/L, corresponding to a blank contribution of 0.08% of the total iodine measured in SRM 3668 and was used to correct the sample measurement data.

All samples were measured by monitoring ^{127}I using inductively coupled plasma mass spectrometry (ICP-MS) on a Thermo X7 system (software build, 2.3.0.161) operating in regular mode. The ICP-MS was tuned and optimized using a standard 1 ng/g multi-element tuning solution. Quadrupole MS routine methods utilize peak jumping, with each of five replicate runs consisting of 150 sweeps (10 s acquisition time), and a dwell time of 30 ms.

The working ^{127}I calibration stock solution was prepared by gravimetric dilution of a high-purity primary standard (SRM 3180, Iodide Anion (I^-) Standard Solution). External calibration curves were constructed using SRM 3180 dilutions. A first order linear fit was applied to the data. The slope and intercept that form the calibration curve was based on the measured ^{127}I response from SRM 3180, and utilized to calculate the mass fraction of iodine in the urine samples.

UI concentrations were not corrected by creatinine for several reasons. The nephritic system of alligators lacks a loop of henle found in mammals, and unlike the saltwater crocodile (*Crocodylus porosus*), there is little osmotic regulation by the cloaca of American alligators exposed to saltwater (Moore et al., 2009; Pidcock et al., 1997). A subsample of alligator urine from this study was tested for creatinine using ELISA techniques (Cayman Chemical