Fecal C ic e ne A e men in he E a le e Sha k, *H*

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ABSTRACT The present study examined the feasibility of measuring the steroid hormone corticosterone in fecal extracts of epaulette sharks, *Hemiscyllium ocellatum*. Six immature, captive-raised epaulette sharks (four females and two males) were obtained from two different zoos and were maintained in a closed-system, 530–liter aquarium. After a one-month adaptation, fecal samples were collected daily from each animal for 33 days. Five-day sets of samples were pooled within animals to insure sufficient material for analysis. Fecal hormone extraction was achieved using repeated cycles of dichloromethane and aqueous washes. The levels of corticosterone were measured by reverse-phase high-performance liquid chromatography (HPLC). Corticosterone presence in HPLC eluent peaks from fecal extracts was determined by comparison of the elution pattern of corticosterone standard with the elution patterns of fecal extracts with and without the addition of tritiated corticosterone or exogenous, unlabeled corticosterone. Exclusive presence of corticosterone in HPLC eluent peaks presumed to be corticosterone was determined by nuclear magnetic resonance mass spectrometry. Corticosterone levels, calculated from a 10–point standard curve, ranged from 1.2 to 20.9 ng/g feces across all sharks, with 92.3% of values being $\leq$13.5 ng/g. Within individuals, the lowest average for corticosterone levels across 33 days was 2.6 ± 0.4 ng/g feces, and the highest average was 8.4 ± 2.2 ng/g feces. This study demonstrated that corticosterone was extractable from and reliably measurable in fecal extracts of epaulette sharks. This is the first evidence of this hormone in epaulette sharks and the first report of fecal corticosterone in elasmobranchs. *J. Exp. Zool. 299A:188*

dependent epaulette shark (*Hemiscyllium ocellatum*).

MATERIALS AND METHODS

This study was performed under auspices of animal-use protocol (IACUC #100679) at the Medical College of Ohio. The epaulette shark is a carnivorous species found off the coast of Australia and New Guinea (Dingerkus and DeFino, '83; Dingerkus, '91; Last and Stevens, '94; Heupel and

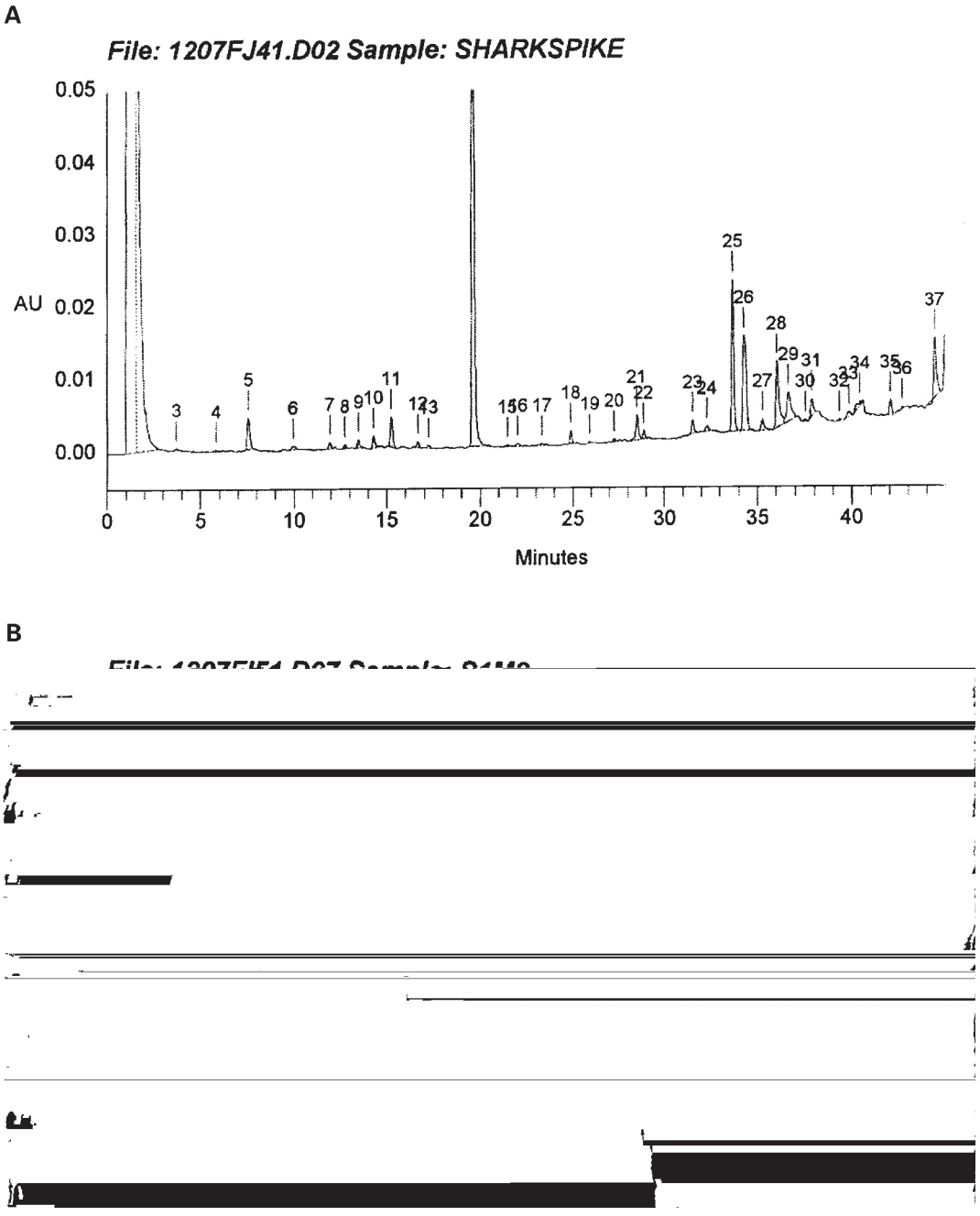

Fig. 1 HPLC elution profiles of two representative epaulette shark fecal extracts. Corticosterone eluted at 19.6 min, which is peak #14 in each sample. Sample A extract was spiked with 1 μg of corticosterone standard, and Sample B extract was unspiked.

extract was $<0.001\%$ of that for the 329.2 peak in the standard.

In the biodegradation test of fecal samples employing a tritiated-corticosterone spike of a pooled fecal sample, radioactivity in the HPLC eluent peak for corticosterone was 5877 CPM and 6401 CPM at zero and 12 hrs of incubation, respectively. These values differed by $<10\%$.

Among all samples collected from all six sharks during the 33–day sampling period, corticosterone ranged from 1.2 to 20.9 ng/g feces (Fig. 4). Values above 13.5 ng/g feces were observed in only 7.7% of samples. The greatest range of within-individual hormone concentration (5–day averages) across the 33–day collection period was from 2.0–20.9 ng/g feces and the smallest range was 1.7–4.5 ng/g

feces. The mean hormone concentration (ng/g feces) within a given individual (i.e., across time) was: female A=7.9

DISCUSSION

The present study has established the presence

(unpublished) suggest that corticosterone levels are unrelated to stress in bonnethead sharks and Atlantic stingrays from the wild. Whether corticosterone is an indication of stress in the epaulette shark remains unknown. However, this study demonstrates the use of fecal casts in the detection of corticosterone, as long as the interference of 1α-hydroxycorticosterone can be ruled out. The fractionation-pattern data in Figure 2B for the fecal-extract eluent (30–sample pool) revealed

15.6–fold range in this regard. Since the fecal hormone response to stress was not addressed in this study, it is not known whether the baseline hormone variability observed could mask the detection of a corticosterone response to stress. However, fecal corticoids in mammals have been shown to increase more than 25–fold in response to ACTH challenge in some species (Wasser et al., 2000), and average 10–fold increases in response to restraint and translocation have been reported (Palme et al., 2000; Turner et al., 2002). While it is possible that the sharks did not adapt fully to the laboratory condition, they had been captive-raised and showed no visible signs of stress during the study. Thus, it seems reasonable that the hormone levels obtained (Fig. 4) represented a minimal-stress or unstressed condition. On the basis of the results of the present study, further studies to determine possible fecal corticosterone response to stress in sharks appear warranted.

This is the first report of corticosterone presence in epaulette sharks and the first use of feces to measure corticosterone in an elasmobranch. It offers the possibility for assessing the status of stress-related steroid hormones in captive elasmobranchs by non-invasive means without handling. It is not known whether fecal hormone measurement will have application in free-ranging benthic elasmobranchs. For non-benthic deep-water elasmobranchs, access to fecal samples is unlikely.

In summary, this study has shown that remote collection of intact fecal casts from epaulette sharks is feasible, and that corticosterone can be measured in these feces using organic extraction and HPLC analysis. This study also provides baseline data for fecal corticosterone levels in six individual sharks across 33 days.

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