

**Research Activity Report**  
**Supported by “Leading Graduate Program in Primatology and Wildlife Science”**  
 (Please be sure to submit this report after the trip that supported by CETbio,PWS.)

	2018. 11, 27
<b>Affiliation/Position</b>	National Institute for Amazonian Research/Research Assistant
<b>Name</b>	Natsumi Hamada Fearnside

<b>1. Country/location of visit</b>
Japan, Kyoto
<b>2. Research project</b>
Genome Science Course. Relationship between behavior and sex hormone concentration in wild yaku sika deer
<b>3. Date (departing from/returning to Japan)</b>
2018. 12. 03 – 2018. 11. 16 and 2018. 12. 19 – 2018. 11. 20 (7 days)
<b>4. Main host researcher and affiliation</b>
Dr. Kinoshita, Assistant Professor at Wildlife Research Center, Kyoto University
<b>5. Progress and results of your research/activity</b> (You can attach extra pages if needed)
Please insert one or more pictures (to be publicly released). Below each picture, please provide a brief description.
<p>We had a meeting on Nov. 12<sup>th</sup> in the afternoon, where professor Kinoshita gave us an overview of what we would be doing, as well as a background on what had been already observed for the sika deer in previous studies. She explained how the extraction process works and how we measure hormone concentration using an enzyme immunoassay. After that, we organized the samples into plastic bags, measured their wet weight and lyophilized them overnight.</p> <p>Nov 13<sup>th</sup> – we weighed the dried samples to assess water loss (and thus, sample freshness) and measured out 0.10g of each sample, which we extracted hormones from by adding an organic solvent (80% methanol) and homogenizing it with beads. The supernatant was then collected to measure hormone concentration.</p> <p>Nov. 14<sup>th</sup> – we did the second plate coating for the enzyme immunoassay for all of the samples (the first plate coating had already been done by professor Kinoshita the previous day), as well as created the standards through serial dilutions of known amounts.</p> <p>Nov. 15<sup>th</sup> – we measured the estrogen and testosterone concentrations by comparing the absorbance of each sample with a concentration curve calculated with the known standards. We then organized the data in a spreadsheet and tested the differences for statistical significance.</p> <p>Nov. 16<sup>th</sup> – we continued analyzing the data, as well as created graphs and charts. We then presented them to professor Kinoshita and professor Sugiura and discussed the results. Though none of the analysis showed statistical significance, a general trend could be observed in some cases. The lack of statistical significance might be due to short sampling time and low n of certain groups such as “males” and “marked females with no fawn”. After, we started writing the poster that was to be presented on the Nov. 20<sup>th</sup>.</p> <p>Nov 19<sup>th</sup> – we finalized the poster and printed it out.</p> <p>Nov 20<sup>th</sup> – the poster was presented at the 9<sup>th</sup> International Seminar on Biodiversity and Evolution.</p> <p>This course was a great opportunity for me to learn more about hormone analysis. It was very educational and I learned a lot about this topic, which I had been wanting to get hands on experience. I am very interested in how hormones affect behavior, and this research allowed me to see firsthand how these analyses are made and what are some of the difficulties and particularities of these tests. I feel like now I have a better view of what type of questions can be answered using fecal samples, as well as the effect of time and hormone stability on the concentrations measured. It showed me that, despite some limitations, fecal samples can be a good source of data for sex steroid hormone analysis, and is particularly useful since it is a non-invasive sampling method. I hope to use this technique in a future’s master’s project.</p> <p>I am currently writing the scientific report with the other group members.</p>

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Fig. 1 – Students weighing fecal samples for hormone analysis in the laboratory.



Fig. 2 – Professor Kinoshita showing students how the concentration curve is calculated using the standards of known concentrations.

## 6. Others

I would like to thank JSPS and PWS for inviting me to take this course and sponsoring its expenses. This has been an incredibly enriching experience for both my professional and personal development and I am very grateful for the opportunity to participate in it.