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## Assessment of fecal glucocorticoid metabolites of cortisol and corticosterone in captive sea otters, *Enhydra lutris*

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# Assessment of fecal glucocorticoid metabolites of cortisol and corticosterone in captive northern sea otters (*Enhydra lutris kenyoni*)

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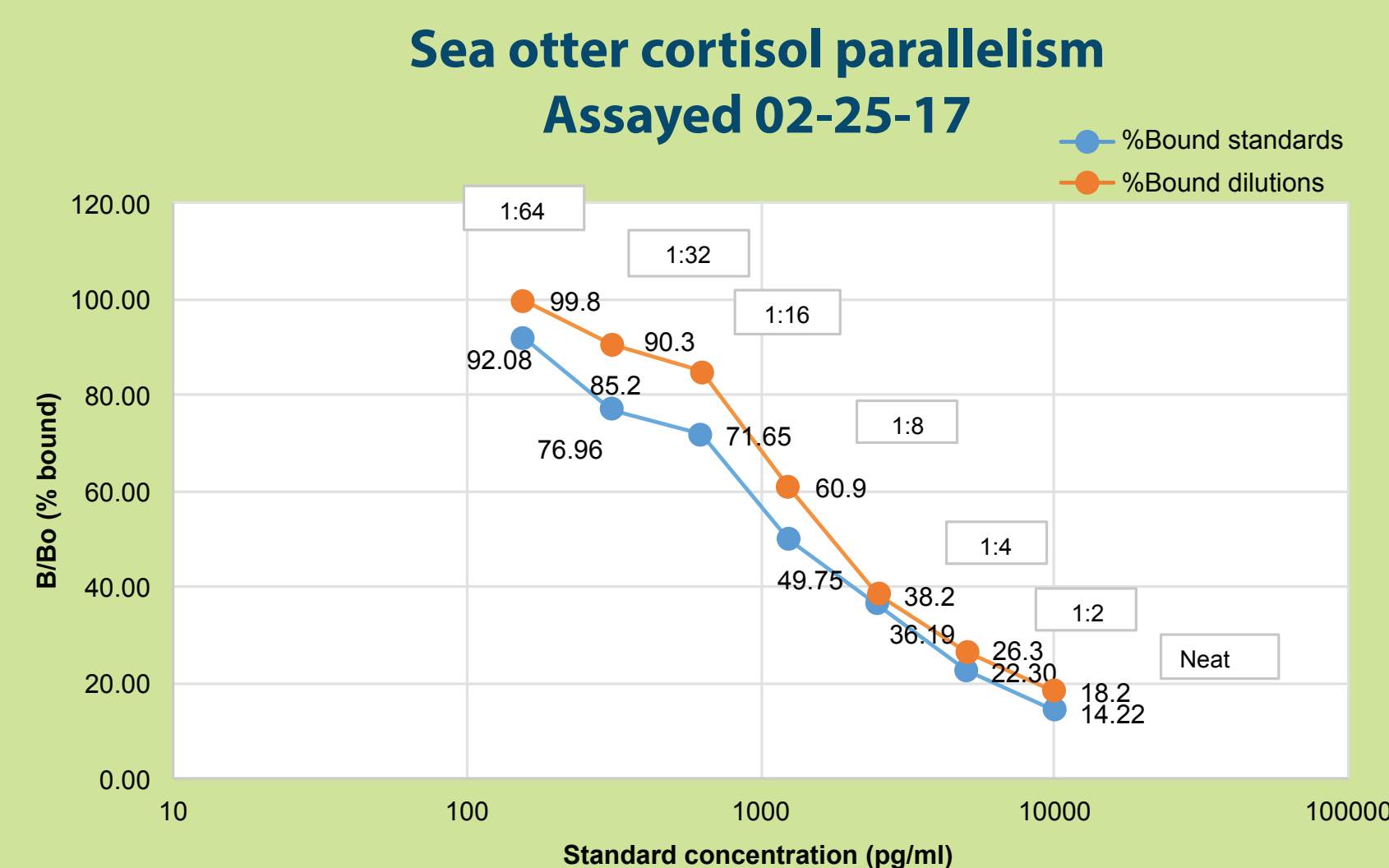
## ABSTRACT:

- The adrenal glucocorticoid hormones, cortisol and corticosterone, are frequently measured in serum as indicators of the vertebrate stress response.
- Sea otters (*Enhydra lutris*), common in zoos and aquariums, have been documented to secrete both cortisol and corticosterone from adrenal glands. These hormones are secreted, metabolized and excreted in feces.
- The goal of this study was to validate the use of non-invasively collected fecal samples to determine baseline metabolite levels for individual sea otters based on sex and age class.
- At the Seattle Aquarium, hormone metabolite groups were measured using commercially available ELISA kits, and validation was achieved by standard parallelism and accuracy tests.
- Differences between animals indicated individual responses to stimuli and peaks above baseline for each animal indicated specific adrenal responses to external stressors.
- Monitoring baseline adrenal hormone levels and events that cause peak values may provide insight for the husbandry management of captive animals to mitigate elevated baseline stress levels and unnecessary external stressors to improve the overall welfare of these marine mammals.

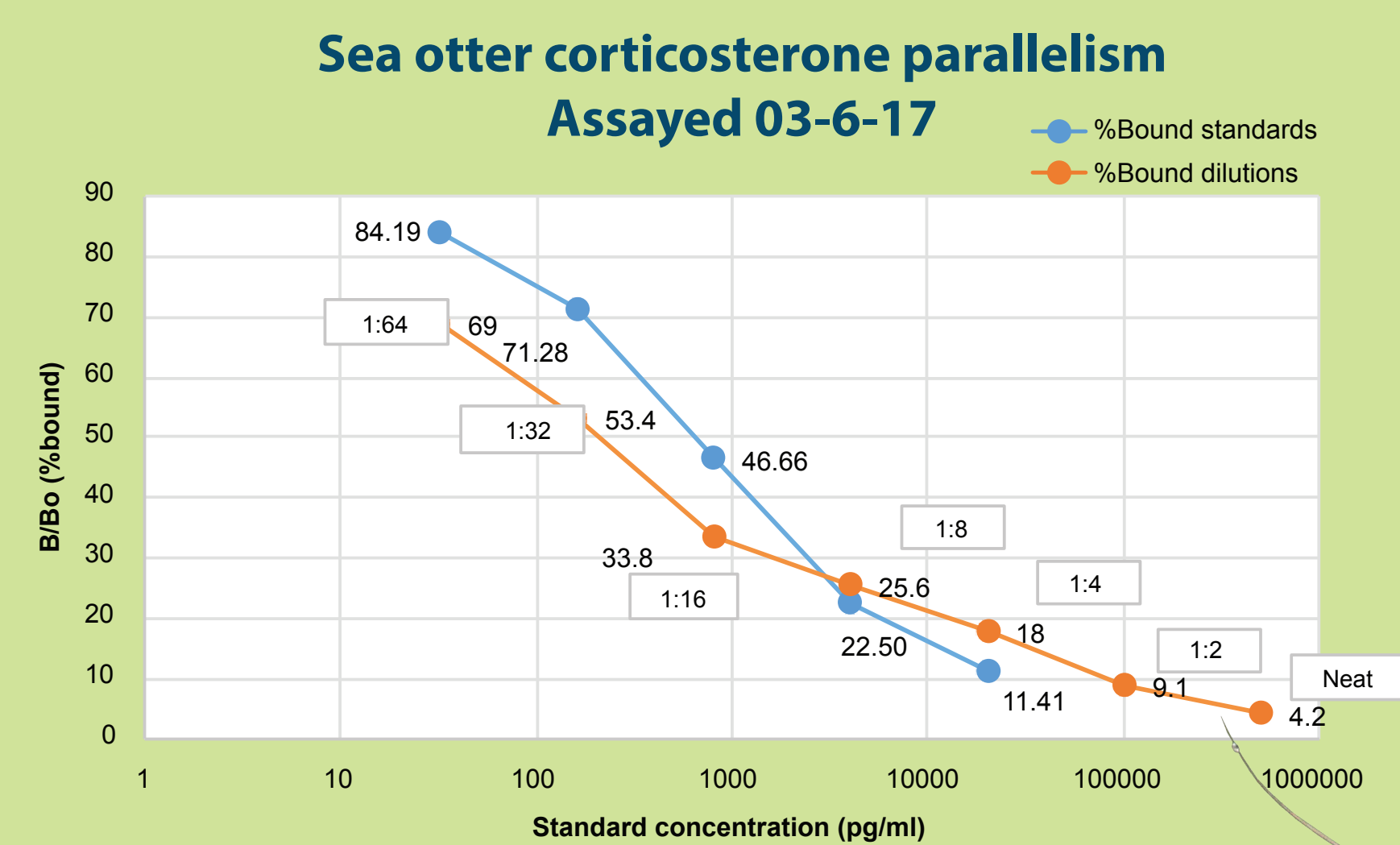
## METHODS:

- Methanol extracted sea otter fecal samples from the Seattle Aquarium:
  - Three female northern sea otters, *Enhydra lutris kenyoni*
  - One male northern sea otter, *Enhydra lutris kenyoni*
- Commercially available ELISA kits for cortisol and corticosterone (Enzo Life Sciences Inc., New York, USA).
- Validation
  - ACTH challenge (Wasser et al., 2000)
  - Parallelism
  - Accuracy

## RESULTS:

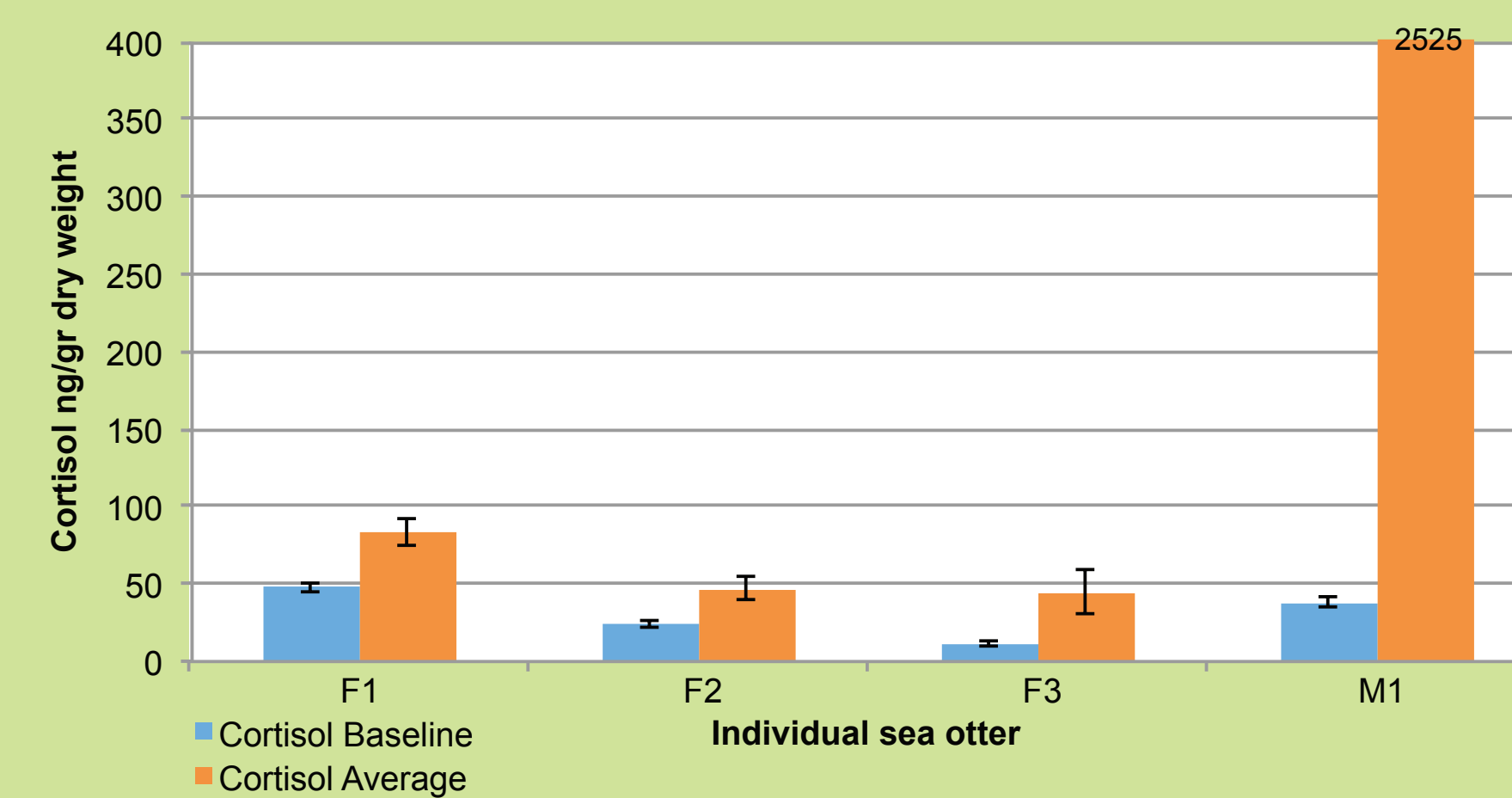


**Figure 1:** Parallelism graph for cortisol metabolites in sea otter feces. Serially diluted standards are graphed with diluted extracts.



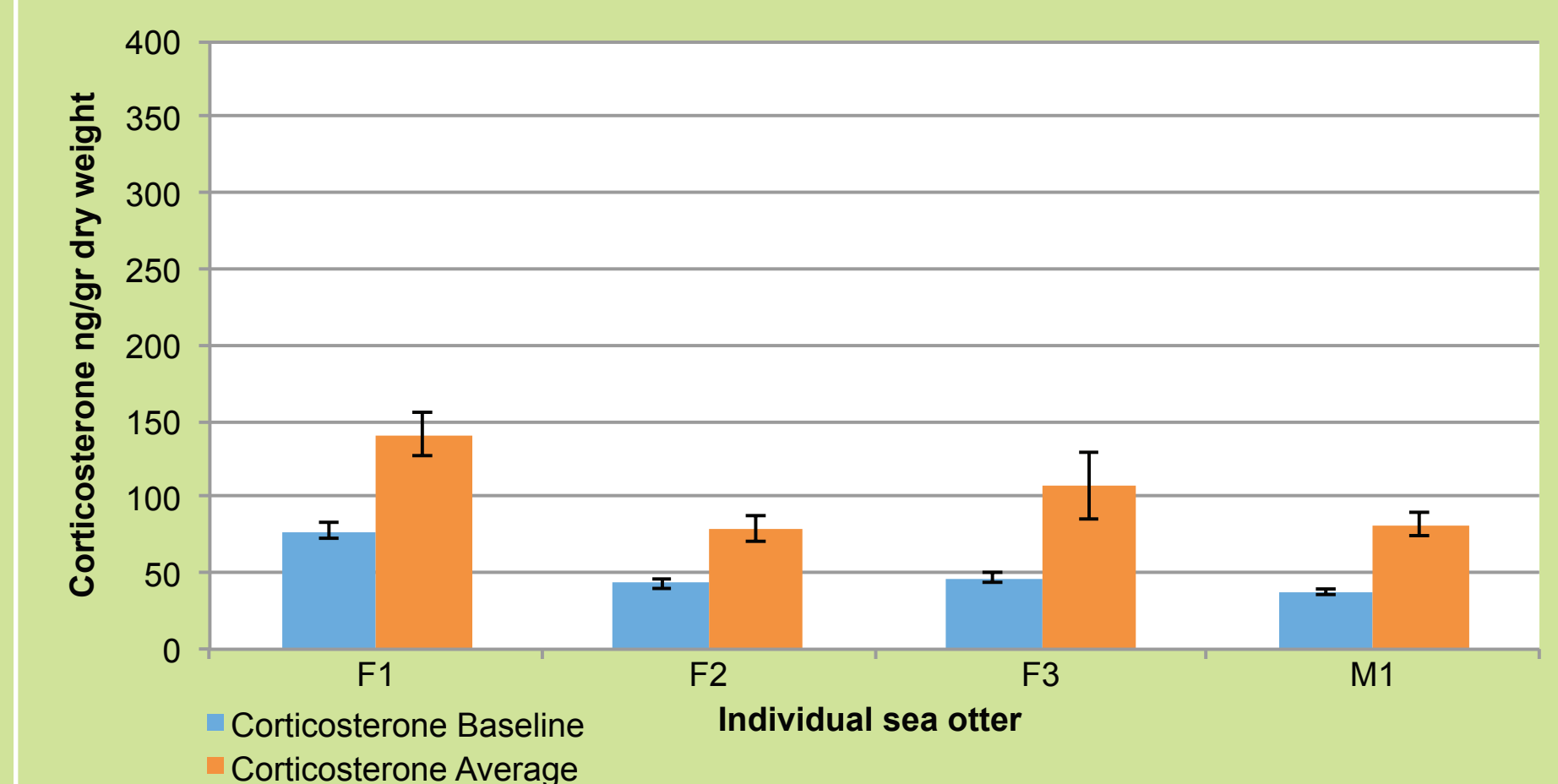
**Figure 2:** Parallelism graph for corticosterone metabolites in sea otter feces. Serially diluted standards are graphed with diluted extracts.

## Cortisol in sea otters



**Figure 3:** Cortisol metabolites (ng/gr dry weight) in four sea otter individuals. Baseline was calculated by removing peaks two times above the standard error and averaging remaining values (blue). Overall average values were calculated with all data points (orange). F1, F2 and F3 are female northern sea otters, and M1 is a male northern sea otter. M1 is an adult male that is housed with a group of females of varying ages and is characterized by keepers as a naturally nervous animal. Overall, baseline cortisol values were below 48 ng/gr dry weight.

## Corticosterone in sea otters



**Figure 4:** Corticosterone metabolites (ng/gr dry weight) in four sea otter individuals. Baseline was calculated by removing peaks two times above the standard error and averaging remaining values (blue). Overall average values were calculated with all data points (orange). F1, F2 and F3 are female northern sea otters, and M1 is a male northern sea otter. M1 is an adult male that is housed with a group of females of varying ages and is characterized by keepers as a naturally nervous animal. Overall, baseline corticosterone values were below 78 ng/gr dry weight.

**Table 1:** Sea otter subjects used for the study. Significant peaks were defined as two times above the standard error of the mean. Samples with peaks in both glucocorticoid metabolites were counted. All peak values were compared to archived daily husbandry notes taken by keepers. The majority of peaks had no significant note, however the \* indicates an event note that is not part of typical activities (training, being weighed, enrichment given).

| Animal ID | Sex    | Age sampled (years) | # Samples tested | # Significant cortisol peaks | # Significant corticosterone peaks | Samples with peaks in both GCs | # Samples with associated notes | Type of note                            |
|-----------|--------|---------------------|------------------|------------------------------|------------------------------------|--------------------------------|---------------------------------|---|
| F1        | Female | 1-19                | 123              | 3                            | 4                                  | 0                              | 5                               | Training, parturition*, weighed         |
| F2        | Female | 1-14                | 73               | 19                           | 21                                 | 9                              | 0                               | None                                    |
| F3        | Female | 1-2                 | 60               | 8                            | 9                                  | 8                              | 3                               | Transport*, enrichment                  |
| M1        | Male   | 7-17                | 233              | 19                           | 63                                 | 2                              | 13                              | Weighed, mating, behavioral, enrichment |

## DISCUSSION:

- Commercially available ELISA kits were validated for cortisol and corticosterone hormone metabolites in sea otter fecal samples.
- There was variation between individuals, indicating differences in individual stress response to stimuli (baseline levels of cortisol ranged from 12 to 48 ng/gr and corticosterone ranged from 30 to 78 ng/gr).
- All three females showed significantly different baseline levels of cortisol and corticosterone, while the male had the same baseline value for both glucocorticoid metabolites.
- Archived husbandry notes from biologists did not correlate to all hormone peaks, so future work should focus on diligent and detailed behavioral and environmental notes to provide a continuous record to better match hormone spikes to stimulatory events.

## ACKNOWLEDGEMENTS:

We would like to thank the staff and volunteers at the Seattle Aquarium who participated in the collection of fecal samples and behavioral data for this study. Special thanks go for their support and assistance. This research was funded by the Seattle Aquarium.

