Investigating the stress response to caging, prey switching, & feeding frequency in the harbor seal (*Phoca vitulina*)

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Introduction

As climate change progresses, the potential for it to impact marine mammals grows, specifically its impact on prey sources for aquatic carnivores like the harbor seal (*Phoca vitulina*). Harbor seals are opportunistic predators whose stomach contents have been shown to include pacific herring (*Clupea pallasii*), walleye pollock (*Gadus chalcogrammus*), and various cephalopod species (Jemison 2001). As ocean temperatures rise due to global warming, the effects could be highly detrimental to populations of harbor seals, which have been steadily declining in recent decades (Pitcher 1990). Ocean warming can cause prey species to shift locations northerly, reducing the potential for harbor seals to utilize that prey source. In response, harbor seals may either expend more energy searching for that prey or switch to a different prey source, one which may not be as nutritionally beneficial. In addition, human endeavors such as commercial fishing have been reducing prey availability. As a result, glucocorticoid levels may rise and the overall health of the animal may be reduced due to nutritional stress. The purpose of this study was to examine the viability of using fecal glucocorticoids, specifically cortisol, for analysis as well as its response to: 1) the effects of caging subjects, 2) the effects of prey switching between lipid-rich herring and lipid-poor pollock (Fig. 1) vs. mixing diets, and 3) the effects of feeding frequency (high vs. low).

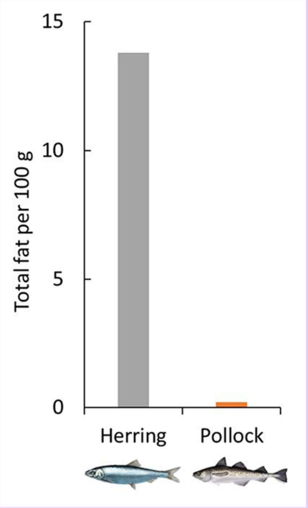

Fig. 1 Difference in fat content between species of harbor seal prey (USDA 2016)

	Year 1			Year 2		
	Season 1 (Sept-Dec)	Season 2 (Jan-Apr)	Season 3 (May-Aug)	Season 1 (Sept-Dec)	Season 2 (Jan-Apr)	Season 3 (May-Aug)
Cecil	Pollock	Herring	Pollock	Herring	Pollock	Herring
Travis	Herring	Pollock	Herring	Pollock	Herring	Pollock
Tina, Snapper	Mixed	Mixed	Mixed	Mixed	Mixed	Mixed
	4x | 1x	4x | 1x	4x | 1x	4x | 1x	4x | 1x	4x | 1x

Fig. 2: Feeding schedule for harbor seals during feeding trials from August 1998 to September 2000. Seals had a 3-month diet acclimation period and were fed at either high frequency (4x) or low frequency (1x) before the 72-hour sampling period.

Methods

Sample Collection:

- Harbor seal scat samples were collected from August 1998 to September 2000 at the Alaska SeaLife Center.
- Seals were fed a diet of either alternating herring and pollock (Cecil & Travis) or a mixed diet (Tina & Snapper) consisting of both for an acclimation period of approximately 3 months (Fig. 2).
- After the 3-month period seals were placed on either a high frequency diet (4x daily) or a low feeding frequency (1x daily).
- Subjects were then caged, and samples were collected over a 72-hour collection period. This was repeated for each of the 6 seasons over a 2-year period.
- Samples were freeze-dried for preservation, grounded into a fine powder, and stored ready for analysis.

Sample Analysis:

- 0.2 g of dried fecal matter is mixed with 2 mL of 90% methanol. The sample is vortexed for 30 min followed by centrifuging for 30 min. The supernatant is extracted.
- The supernatant is diluted with 90% methanol in a 1:6 ratio to bring results within the detectable range (0 – 800 ng/mL) of the Enzyme Linked Immunosorbent Assay (ELISA).
- An ELISA (DRG International Inc. Cortisol ELISA EIA-1887) is used to determine the cortisol level in each sample using standards provided with the ELISA kit, with a sensitivity of 2.5 ng/mL.
- All samples are assayed in duplicate, with the mean cortisol concentration used for data analysis.
- Data collected from the values given by the spectrophotometer (Beckman Coulter, DTX 880) is fitted to a 4-parameter logistical curve and corrected from the 1:6 dilution ratio.

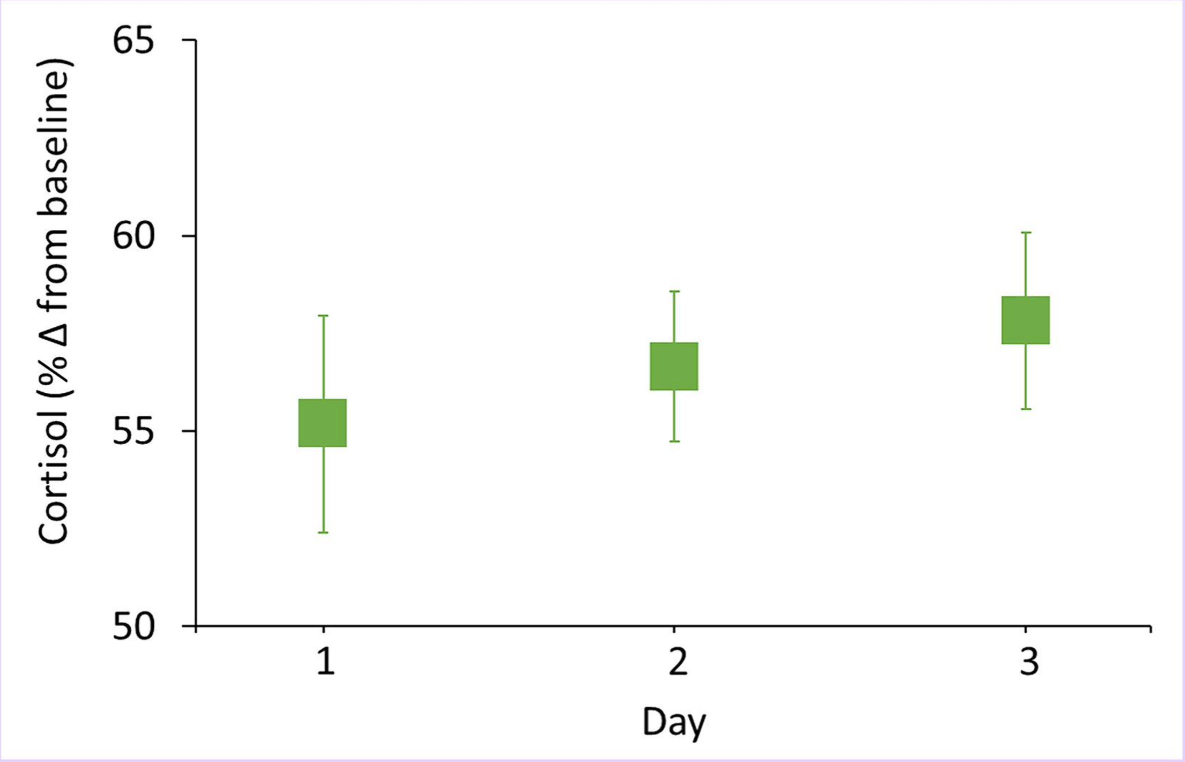

Fig. 3: Average percent change in cortisol from baseline versus day of caging. Each mean value represents pooled scat data from all animals. Error bars indicate ±SE.

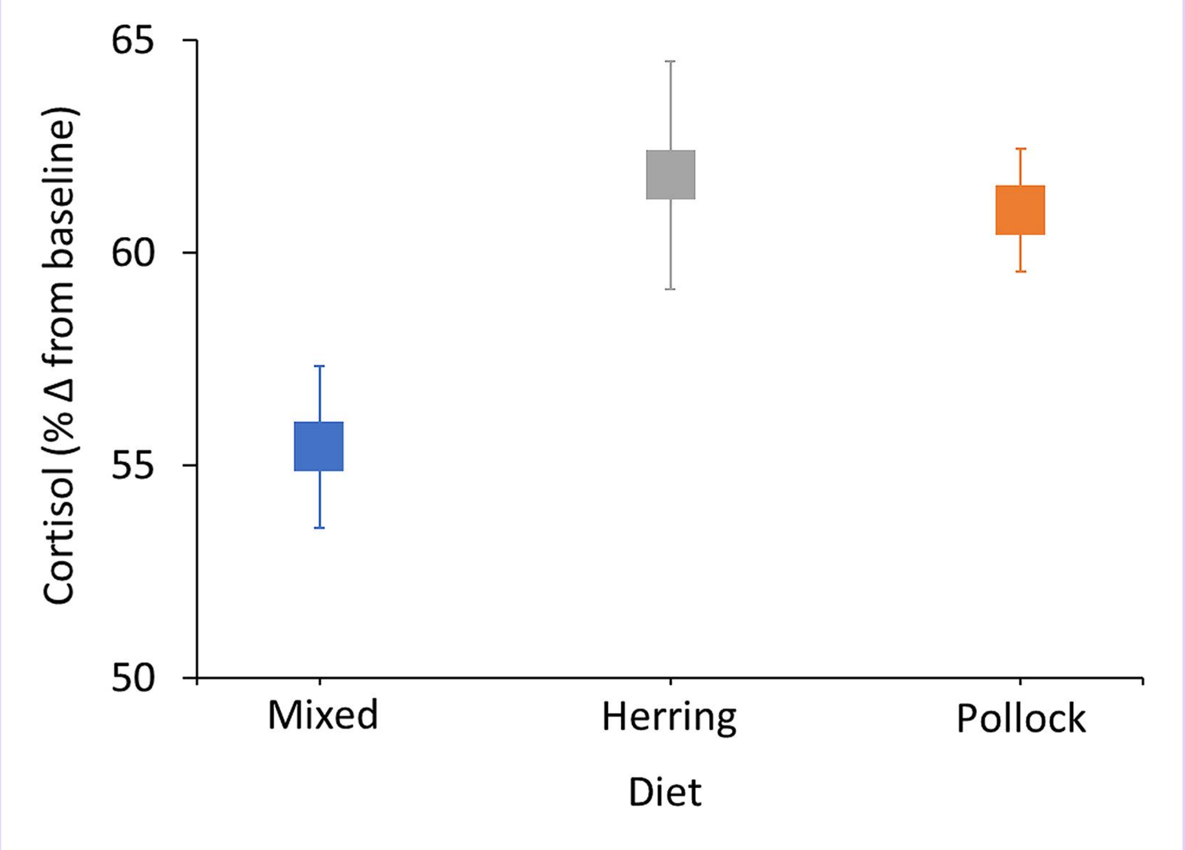

Fig. 4: Average percent change in cortisol from baseline versus type of diet. Each mean value represents pooled scat data from all animals that were fed that respective diet. Error bars indicate ±SE.

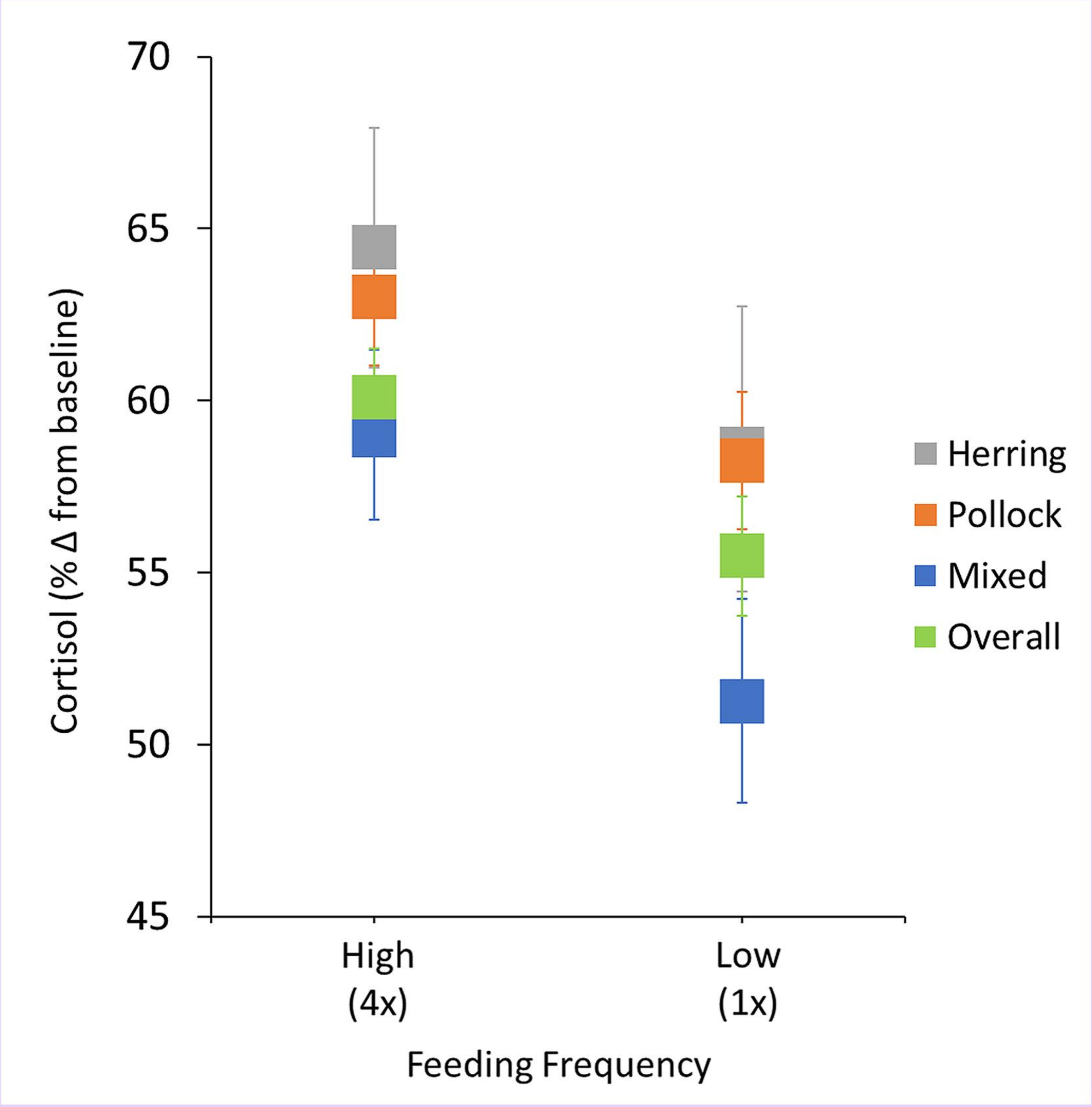

Fig. 5: Average percent change in cortisol from baseline versus feeding frequency. Each mean value represents pooled scat data from all animals. Overall value is the average for each feeding frequency. Error bars indicate ±SE.

Results

- From a total of 4 subjects, 271 samples were analyzed.

Cage Effect:

- Data was pooled from all animals' samples into day 1 (n = 67), 2 (n = 130), and 3 (n = 74) of the caging period (Fig. 3).
- Average percent change of cortisol from baseline for each day:
  - Day 1: 55.18%
  - Day 2: 56.65%
  - Day 3: 57.82%
- One-way analysis of variance (ANOVA) showed no significant difference in cortisol levels between days 1, 2 and 3 ($p = 0.7667$).

Prey Switching:

- Data was pooled from all animals' samples into either herring (n = 58), pollock (n = 92), or mixed diets (n = 112) (Fig. 4).
- Average percent change of cortisol from baseline for each diet:
  - Mixed: 55.44%
  - Herring: 61.82%
  - Pollock: 61.00%
- ANOVA tests showed a significant difference between the diets ($p = 0.0064$).
- Post-hoc Tukey's tests showed a significant difference in cortisol levels between mixed and pollock diets ($p = 0.0054$) but no significant difference between mixed and herring ($p = 0.1547$) or pollock and herring ($p = 0.6504$).

High vs. Low Feeding Frequency:

- Data was pooled from all animals' samples into either high feeding frequency (n = 161) or low feeding frequency (n = 110) (Fig. 5).
- Average percent change of cortisol from baseline for each feeding frequency:

High:	Low:
- Herring: 64.44%	- Herring: 58.60%
- Pollock: 63.01%	- Pollock: 58.26%
- Mixed: 59.00%	- Mixed: 51.27%
- Overall: 60.10%	- Overall: 55.48%

- Two-way t-tests showed a significant difference between high feeding frequency and low feeding frequency rates ($p = 0.0125$).

Discussion

Cage Effect:

- The results showed that there was no change in the stress levels during the 3-day sampling period.
- Therefore, the stress response that was exhibited in relation to diet change and feeding frequency were unaffected by the animals' response to the cage.

Prey Switching:

- Seals on mixed diets had a lower stress response on average than those on pollock diets over the 2-year experimental period.
- It has been shown that digestive efficiency and regulatory functions in harbor seals are affected by the kind of prey they consume, with a diverse, mixed diet being ideal (Trumble and Castellini 2005).
- This can be attributed to the varying composition of fatty acids in different species of prey. Herring has greater amounts of saturated fatty acids while pollock has greater amounts of unsaturated fatty acids (Iverson et al. 2002).
- Saturated and unsaturated fatty acids have different transport mechanisms, and animals have a finite amount of those transporters. With a mixed diet, more transporters can be utilized and therefore digestion is faster and more efficient whereas with a single diet dominated by a single type of fatty acid, less transporters can be utilized and digestion is therefore slower.
- Furthermore, regulatory hormones may be inhibited with a single diet (Trumble and Castellini 2005).
- These factors may result in greater stress during single herring or pollock diets.
- However, the results showed that there was no significant difference in stress between a mixed and herring diet. This is likely due to the differing fat composition of the two single prey diets (Fig. 1). Herring is lipid-rich and is therefore a more nutritious source of prey than the low-lipid pollock.

High vs. Low Feeding Frequency:

- A higher feeding frequency consistently yielded greater stress levels among the seals than a lower feeding frequency, for all diets.
- Previous studies have shown that digestibility in harbor seals decreases as feeding frequency increases (Trumble and Castellini, 2005).
- This may be due to slower gastric movement as a result of greater intragastric volume in a low-lipid diet or greater lipid load in a high-lipid diet (Mateos et al. 1982).
- A balance between volume and mass results in higher digestibility, therefore suggesting why a mixed diet results in less stress than a single pollock and herring diet.

Conclusion

This study showed that fecal glucocorticoids could be extracted and analyzed accurately using an ELISA kit. It was found that there was no increased stress response in harbor seals from caging. In addition, mixed diets on average had a lower stress response than either pollock or herring diets, and high feeding frequencies caused a higher average stress response in harbor seals than low feeding frequencies. This information may shed more light on the rapidly declining harbor seal population in recent years and on our human impact. Climate change has the potential to shift prey species of harbor seals and could result in a reliance on one species of prey instead of having a balanced diet of multiple prey sources. Seals may also be forced to eat at a higher frequency when they come across prey, as prey may become more dispersed due to ocean warming and less abundant due to commercial fishing. This may lead to higher stress levels in harbor seals and lower overall health.

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