

Evaluating competition effects on CORT and body condition in *Plethodon hubrichti*

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Abstract

The Peaks of Otter Salamander, *Plethodon hubrichti*, resides within a 15 km stretch of the Blue Ridge Mountains in central Virginia and is classified as a species of special concern. The Eastern red-backed salamander, *Plethodon cinereus*, has a broad range which overlaps that of *P. hubrichti*. In regions of sympatry, the competitive interactions between the two species may potentially influence the stress levels of the Peaks of Otter salamander. Such stress is earmarked by the release of corticosterone (CORT) which is the primary glucocorticoid released by the hypothalamic-pituitary-interrenal (HPI) axis in amphibians. This research focuses on examining the extent to which *P. hubrichti* experiences stress due to competition with *P. cinereus* through analysis of CORT and the scaled mass index (SMI) collected from Peaks of Otter salamanders in both allopatric and sympatric regions. A non-invasive waterborne technique is used to collect both baseline and agitation CORT concentrations from *P. hubrichti*. The hormone samples are then extracted and plated on an enzyme-linked immunosorbent assay (ELISA) for analysis. It is hypothesized that the baseline CORT for salamanders in allopatric habitats will be lower than their sympatric counterparts. Further, an inability to mount a physiological response to an acute stressor, displayed by lower agitation values, may indicate the salamanders are under chronic stress. Chronically stressed salamanders would suffer from weaker immune systems, lower reproduction, and reduced responsiveness to additional stressors, which lead to poorer health. Preliminary results show higher CORT values in agitation samples than in baseline samples, suggesting a functional HPI axis and no indication of chronic stress. Moreover, preliminary SMI data indicates higher body condition in sympatric sites than in allopatric sites, corroborating the CORT data. Further research will be conducted to process and analyze the remaining hormone samples to assess the validity of the initial results.

Introduction

The peaks of Otter Salamander, *P. hubrichti*, is a Species of salamander endemic to the Blue Ridge Mountains. It has been shown that *P. hubrichti* are negatively affected by lower altitudes, restricting them to their small range of 15 km at elevations above 450m. Nearing the parameters of this range, *P. hubrichti* experience poorer reproduction, worse survival rates, and slower growth rates. *P. hubrichti* prefer a cool, moist, deciduous forest, and utilize a complex system of burrows underground, particularly in the colder winter months. *Plethodon cinereus* also utilize these burrow systems in addition to sharing a near identical diet of invertebrate insects which dwell in leaf litter, such as termites, ants, and mites. These factors support the notion that *P. hubrichti* could be experiencing stress due to competition in the areas where both species ranges overlap. This competition may increase the susceptibility of *P. hubrichti* to habitat loss, and consequently, a threatening decline in population. Areas where *P. cinereus* are found with *P. hubrichti* are deemed sympatric, whereas those in which only *P. hubrichti* are found are regarded as allopatric. When salamanders are stressed, they produce corticosterone (CORT; Dantzer, 2014), the primary glucocorticoid synthesized by the hypothalamo-pituitary-interrenal, HPI axis, in amphibians (Denver, 2009). When an amphibian experiences prolonged stressors at which point they can no longer mount an additional stress response, they are considered chronically stressed. Chronic stress in amphibians has been linked to ineffective reproduction, immunosuppression, increasing likelihood of infection (Kaiser et al. 2015), and heightened metabolic rates, each causing a decrease in overall health of the amphibians. The Scaled Mass Index (SMI) is an additional measure used to obtain a standard value of body condition, indicative of overall health (Peig & Green, 2009). SMI is calculated using both the mass of the salamander and its length from snout to vent. This research seeks to evaluate the extent to which competition acts as a stressor for *P. hubrichti* by analyzing baseline and agitation-induced CORT concentrations from allopatric and sympatric sites. We anticipate higher CORT levels and lower SMI values from sympatric sites due to the effects of competition as a stressor.

Materials and Methods

Each season an allopatric site and a sympatric site are visited twice. At each site, up to 40 salamanders are collected, depending on conditions and surface-active salamander density. As they are collected, they are put in a petri dish with 15 mL of spring water. To establish a baseline, half of the collected salamanders remain stationary in the petri dish for 1 hour. The other half are gently agitated for 1 minute every 3 minutes, over the course of 1 hour, causing a heightened stress response. The produced CORT is collected both as it diffuses into the spring water, and through feces and urine. This non-invasive waterborne sampling technique minimizes ecological effect (Gabor et al. 2016; Navarro, 2018), a method necessary for this species of special concern. The sample is then collected in a conical tube to be analyzed at the lab. The length of the salamander, from snout to vent, is then measured along with its weight to calculate SMI values as an indicator of body condition. Each salamander is then immediately returned to the location in which it was found using a flag marker system. If the samples are not immediately extracted, they are stored at -20° C. The hormones are extracted using solid-phase extraction (SPE) and then concentrated to a powder using a SpeedVac. The samples are then resuspended in a buffer and plated on a 96 well enzyme immuno-assay (EIA) plate for analysis. The plate is read using an absorbance spectrophotometer to produce quantitative value of the CORT concentration from each sample. Statistical analysis of the SMI values and CORT concentrations are performed using f-tests and t-tests to determine statistical significance. CORT concentrations from both allopatric and sympatric sites for the fall 2022 and spring 2023 have been analyzed (Figures 4 & 5). Additionally, the SMI values from all sympatric and allopatric sites have been analyzed and compared across fall 2022, spring 2023, and fall 2023. As this research is still in the early stages, the most recent CORT samples have yet to be analyzed.

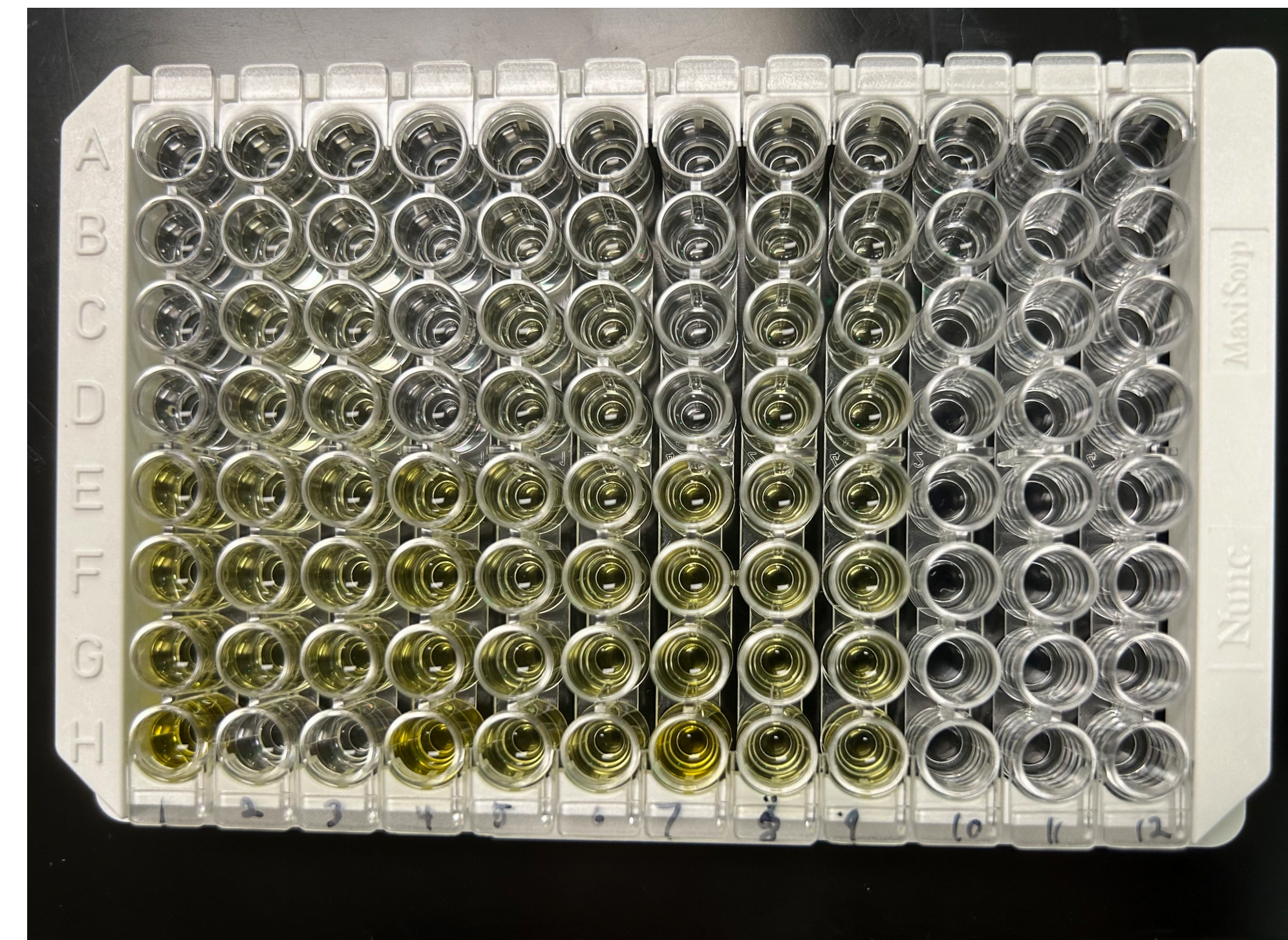


Figure 1. Enzyme-linked immunosorbent assay (ELISA) plate used to measure CORT within a sample. Photo by Jason Porter.

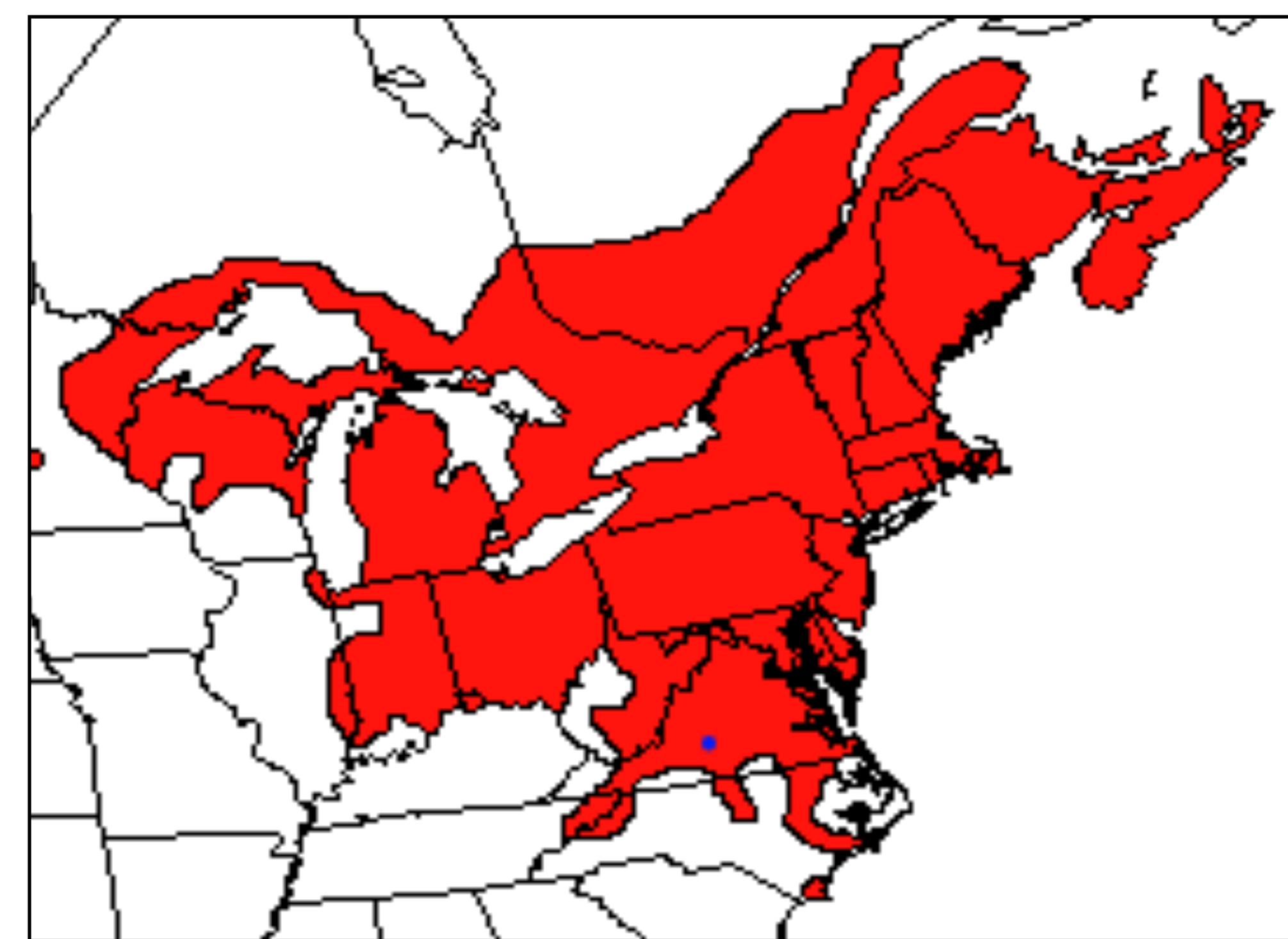


Figure 2. The distribution of the Peaks of Otter Salamander (*Plethodon hubrichti*); blue shaded dot, approximately 15 km along the Blue Ridge Parkway) and the Eastern Redback Salamander (*P. cinereus*; red shaded area).



Figure 3. The Peaks of Otter salamander (*P. hubrichti*) displaying its key identifier of gold/bronze freckling along its dorsum. Photo by Cheyenne Brooks

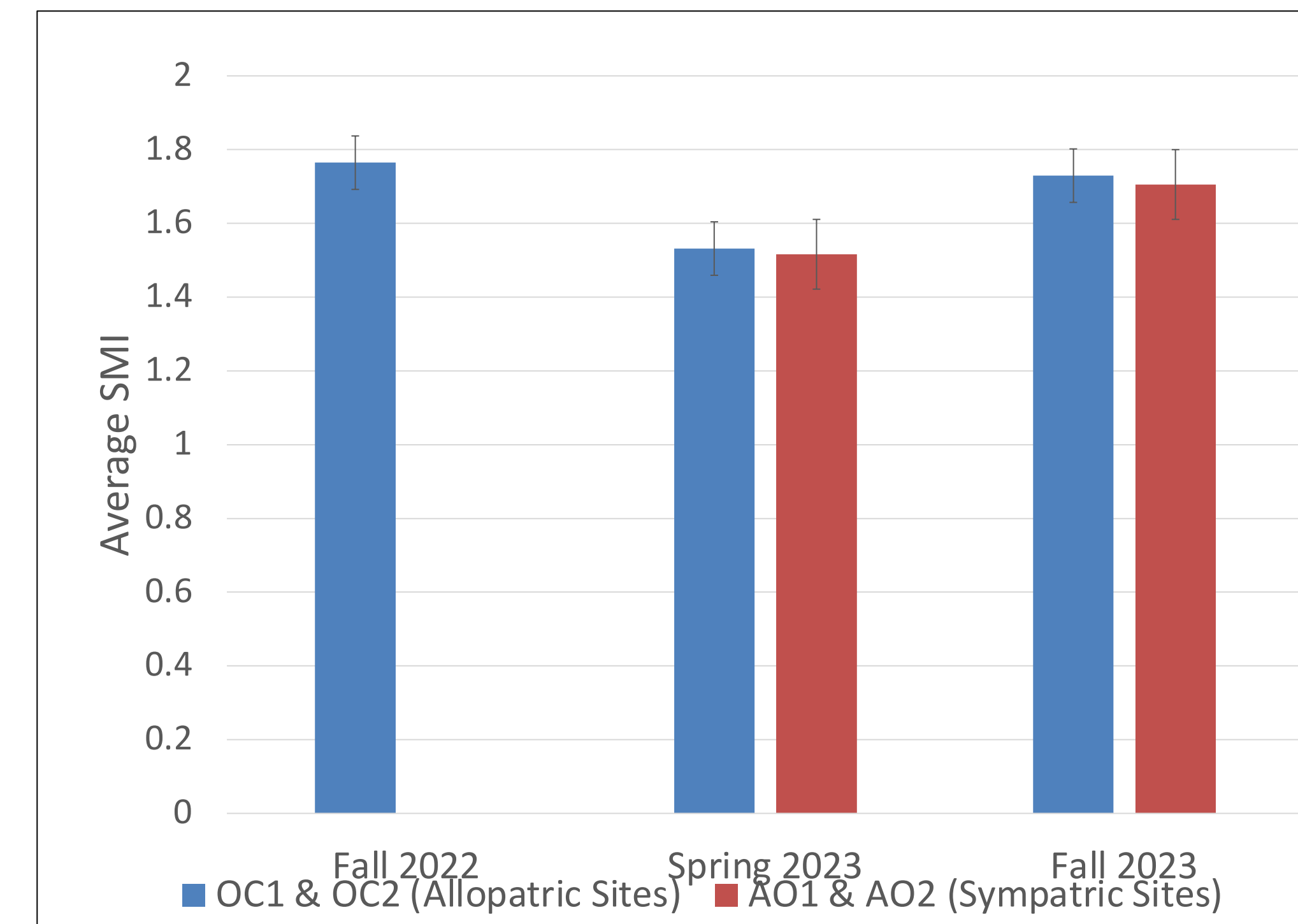


Figure 4. Comparison of average SMI values at allopatric and sympatric sites across three seasons

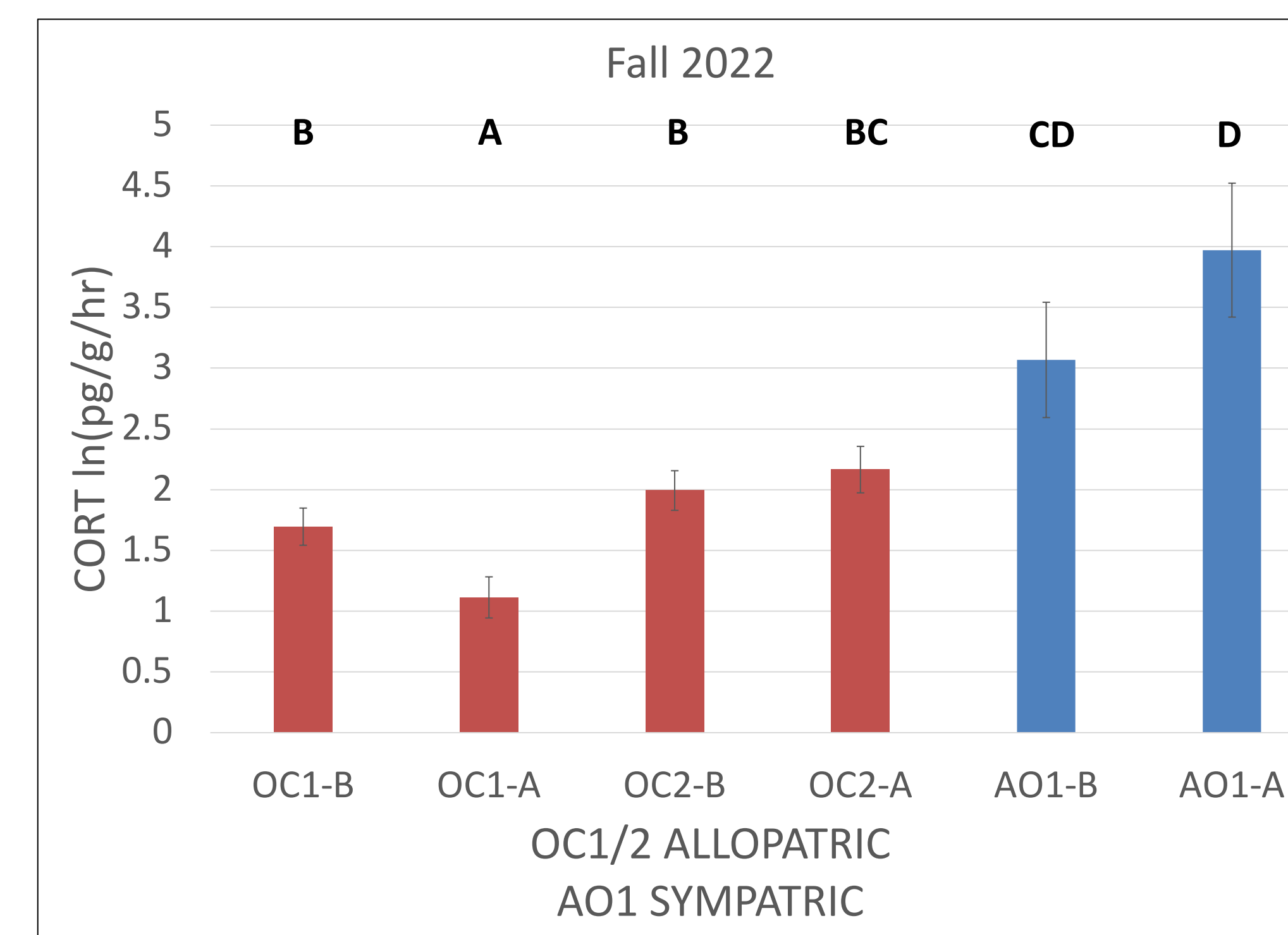


Figure 5. Comparison between CORT levels at allopatric (OC1/OC2) versus sympatric (AO1) sites and baseline (B) versus agitation (A) samples in fall 2022

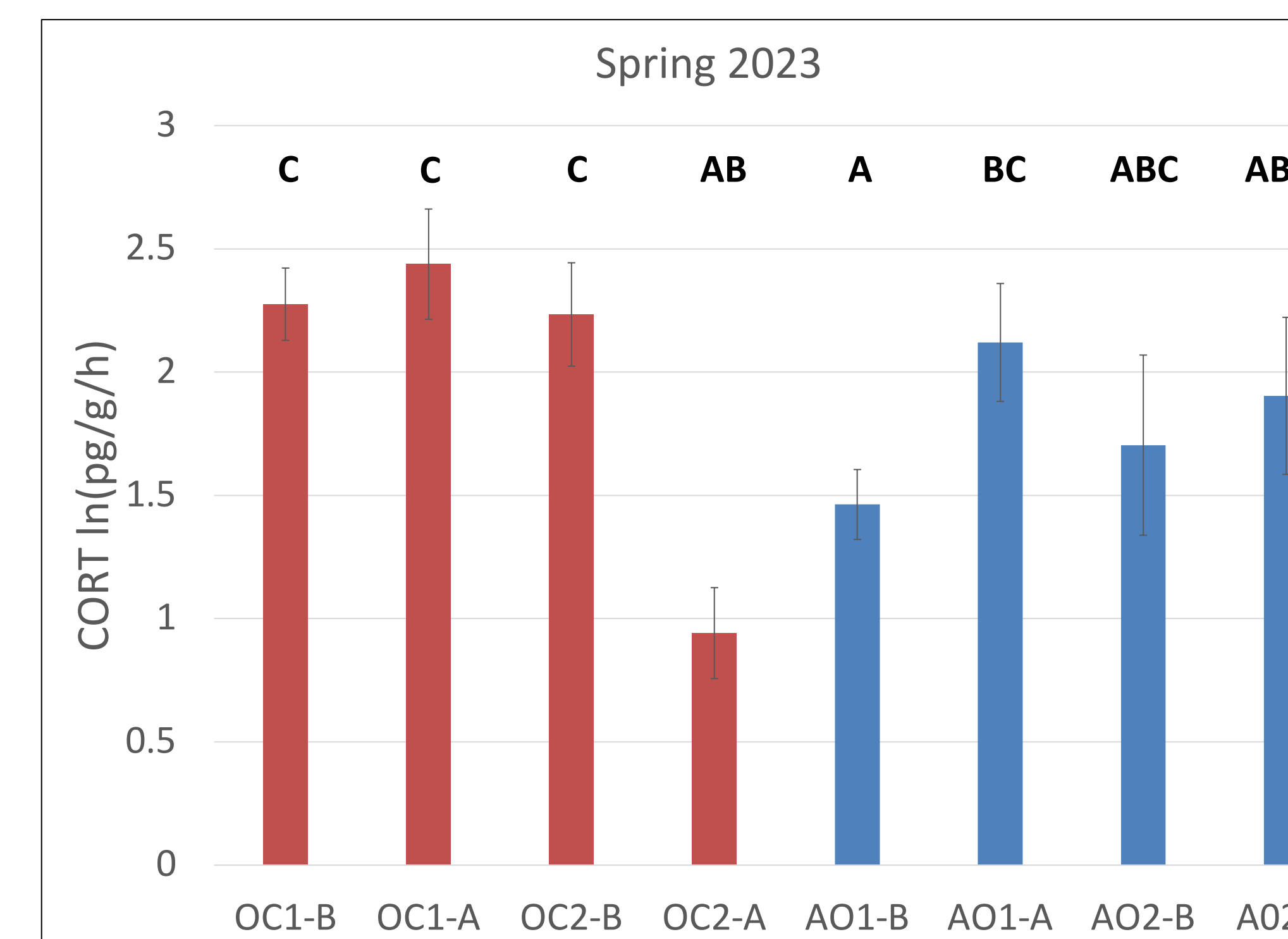


Figure 6. Comparison between CORT levels at allopatric (OC1/OC2) versus sympatric (AO1/AO2) sites and baseline (B) versus agitation (A) samples in spring 2023

Results

1. A comparison between the SMI values of *P. hubrichti* in allopatric sites and sympatric sites yielded no statistically significant difference in the fall 2022, spring 2023, or fall 2023 season.
2. In the fall of 2022 CORT concentrations, OC1-B displayed a significantly higher concentration than that of OC1-A ($t=x$, $df=38$, $p=0.0149$). OC1-B showed significantly lower hormone concentrations than AO1-B ($t=x$, $df=17$, $p=0.0135$) and AO1-A ($t=x$, $df=16$, $p=0.0011$). OC1-A also displayed significantly lower CORT concentrations than each of the other samples, OC2-B ($t=x$, $df=38$, $p=0.0006$), OC2-A ($t=x$, $df=32$, $p=0.0002$), AO1-B ($t=-2.1428$, $df=17$, $p=0.0469$), and AO1-A ($t=-4.9575$, $df=17$, $p=0.0001$). Additionally, OC2-B was significantly lower than that of AO1-B ($t=-2.1427$, $df=17$, $p=0.047$) and AO1-A ($t=-3.4420$, $df=16$, $p=0.0033$). Finally, OC2-A showed a significantly lower CORT concentration than that of AO1-A ($t=-3.0964$, $df=17$, $p=0.0066$).
3. In the CORT concentrations from spring of 2023, OC1-B had a significantly higher value than OC2-A ($t=2.5270$, $df=22$, $p=0.0192$) and AO1-B ($t=3.9840$, $df=38$, $p=0.003$). Similarly, OC1-A showed a significantly higher value than OC2-A ($t=2.71683$, $df=32$, $p=0.0105$) and AO1-B ($t=3.7227$, $df=37$, $p=0.0006$). OC2-B was significantly higher than OC2-A ($t=2.2093$, $df=33$, $p=0.0171$) and AO1-B ($t=3.0451$, $df=38$, $p=0.0042$) as well. The AO1-B sample less concentrated than that of the AO1-A sample. ($t=-2.3638$, $df=31$, $p=0.0245$)

Discussion & Future Work

Contrary to our predictions, SMI did not differ between allopatric and sympatric sites, suggesting the body condition of salamanders at similar elevations may not be affected by competition. The fall 2022 results support the hypothesis that *P. hubrichti* in sympatric locations have greater CORT values than their allopatric counterparts suggesting greater stress. However, the spring 2023 data does not support the same conclusion due to lack of statistical significance between sites. It was also expected that *P. hubrichti* in allopatric locations would be able to mount greater responses to stressors, indicated by increased CORT values in agitation versus baseline samples within a site, than those in competitive environments. However, our current data does not provide any statistically significant results to support this hypothesis. The lack of a difference between baseline and agitation samples may indicate a suppressed response, or even chronic stress. The inconclusive data prompts additional research to more completely evaluate competition effects on CORT and body condition of *P. hubrichti*.

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