

# ASSESSING FECAL STRESS HORMONE METABOLITES LEVELS IN THE SPRINTS OF EURASIAN OTTER *LUTRA LUTRA*

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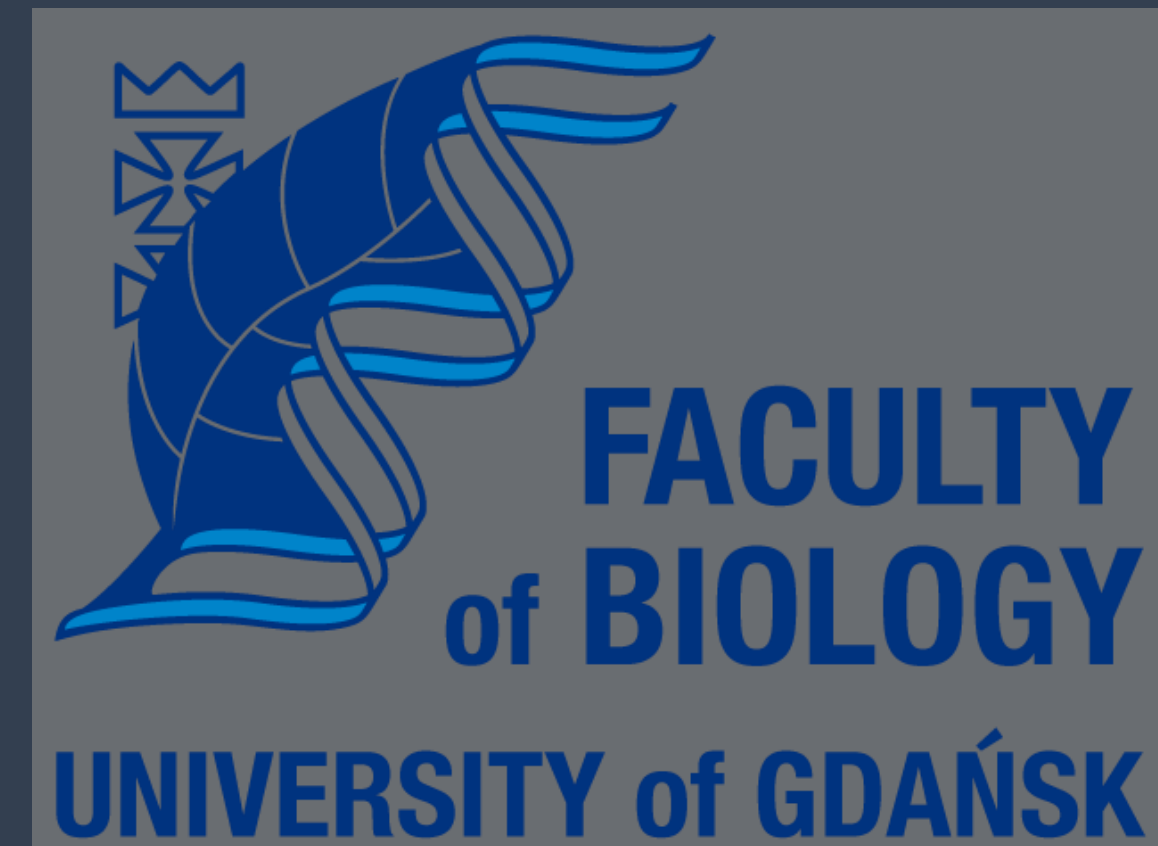
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## INTRODUCTION

Stress hormone analysis is a widely used tool to assess the wellbeing of livestock, wild animals in captivity, and is becoming more popular in wildlife research. Factors like the choice of matrix, method of analysis, time from collecting the sample, conservation and extraction method, all have impact on the final result. We did the first attempt of assessing fecal glucocorticoid metabolites levels of the Eurasian otter, and compared the results with some variables that might influence the stress level in this semiaquatic predator.



## METHODS

Sprints were collected in field, in central Poland, along the Vistula river and smaller rivers nearby, in three different seasons of 2016. Only fresh faeces, not older than 24 hours after defecation, were used. Sprints were frozen during field trip and stored frozen till extraction. The extract was also stored frozen between the analyses. 62 suitable samples have been analyzed as follows:

Subsampling 0,3 g of each wet faeces

extraction in 5 ml of 96% ethanol (hot water bath, 20 min., mixing)

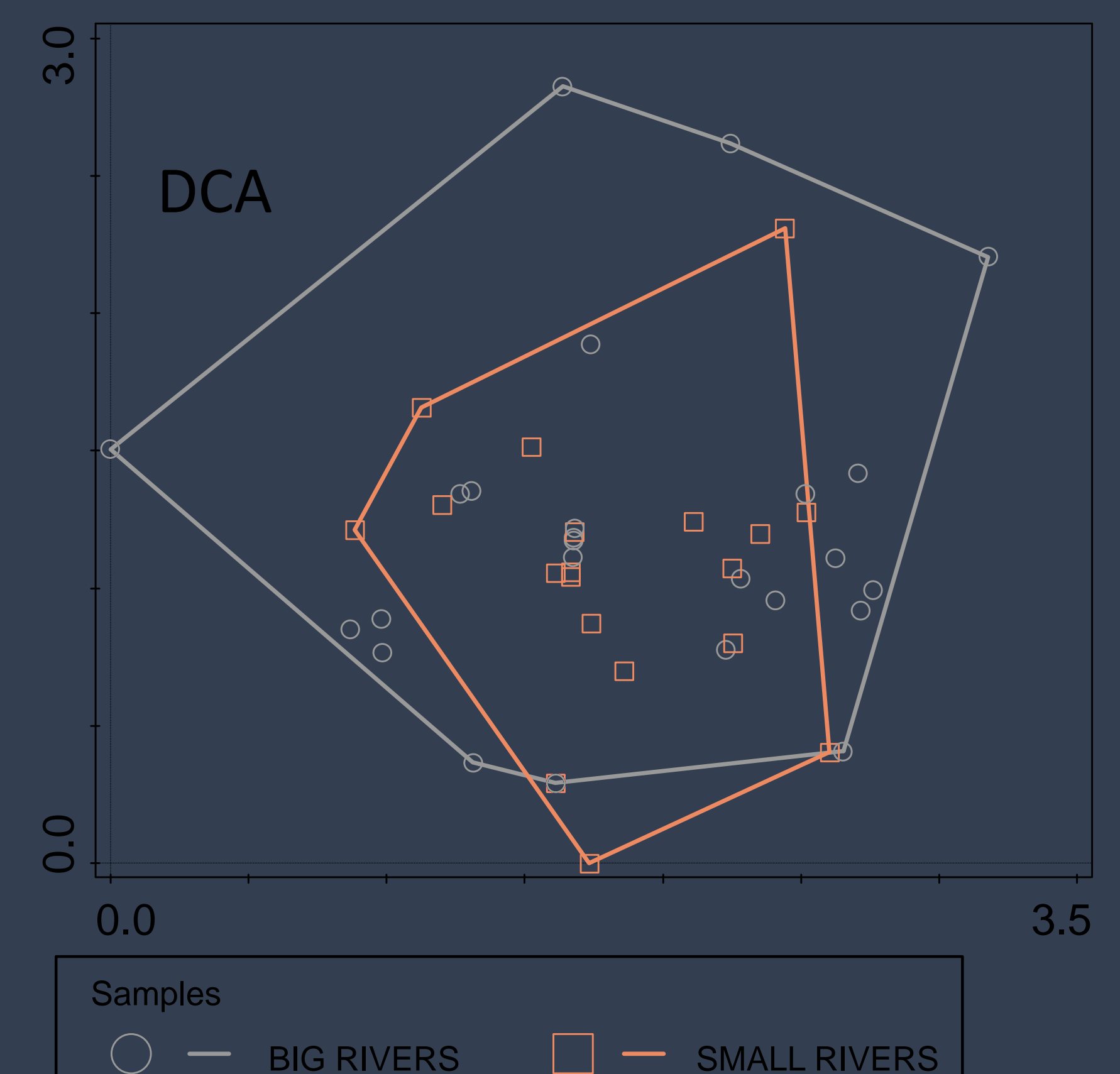
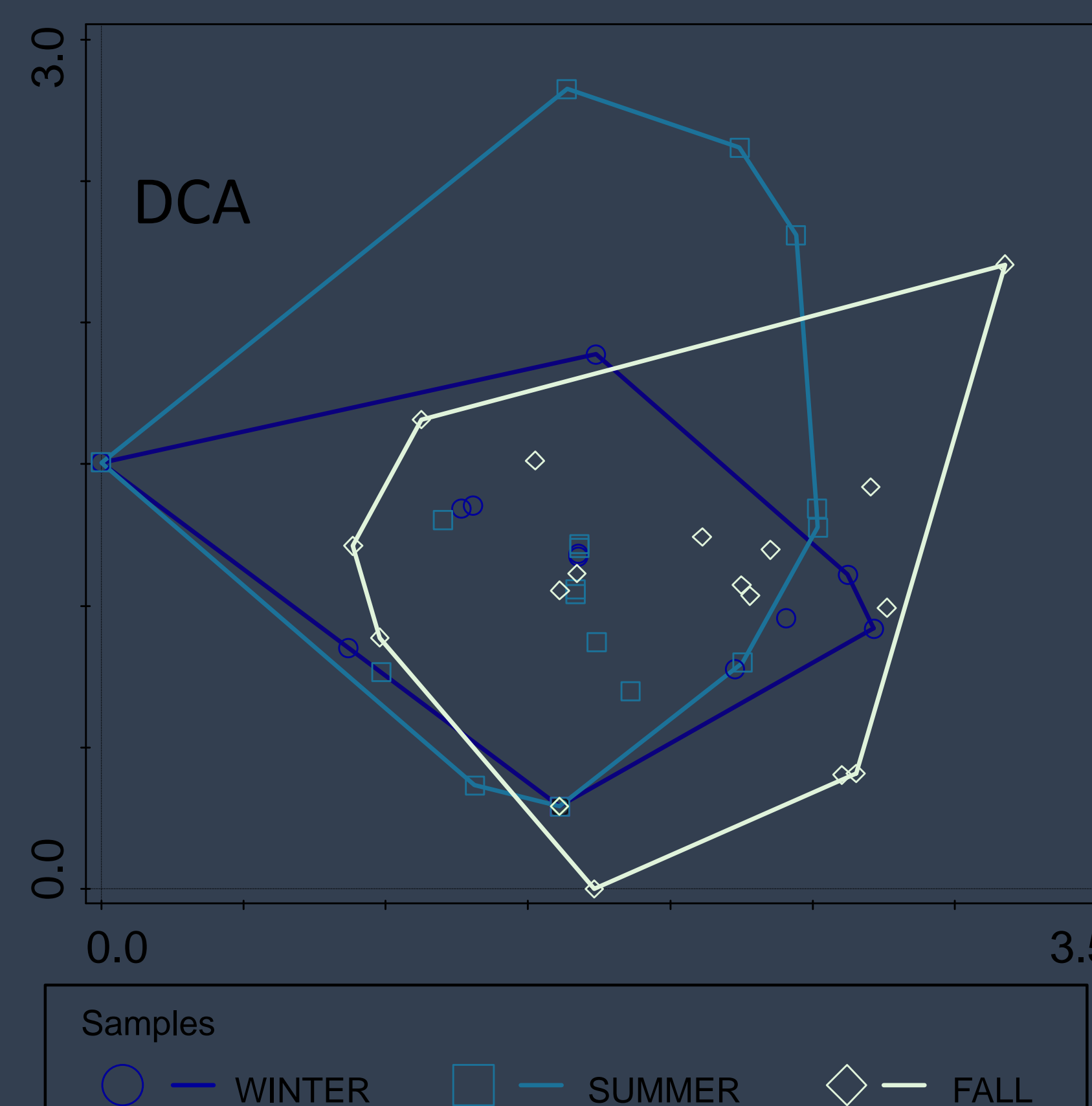
Analysis 1: 100 µl of the extract diluted with 200 µl of methanol  
Analysis 2: 100 µl of the extract

High performance liquid chromatography - mass spectrometry (HPLC-MS)

## RESULTS

Fecal cortisol and corticosterone metabolites were not detected in every sample. Analysis repeated on less diluted samples detected the metabolites in significantly more samples (chi-squared test,  $df=5$ ,  $\chi^2=16,83$ ,  $p<0,01$ ).

The fecal metabolites levels were not dependent on the hour of sample collection (Canonical correspondence analysis - CCA, Pseudo-F=0,7,  $p=0,7$ ). The situation was similar with the undigested material dry weight (CCA, Pseudo-F=0,9,  $p=0,52$ ).



Data obtained during the second approach were used for further analysis. There was the variation in stress levels of otters (Detrended correspondence analysis – DCA, Total variation=1,78). None of the factors taken into consideration so far, such as season, river width, or diet composition explains the variation in the hormone levels (CCA, adjusted explained variation 0%, season: Pseudo-F=0,6,  $p=0,9$ , river width: Pseudo-F=0,8,  $p=0,58$ , diet: adjusted explained variation 4,4% Pseudo-F=1,5,  $p=0,096$ ).



Photo: Zuzanna Pestka

## CONCLUSIONS

- Probably because of the specific structure of otter sprints, which contain relatively big amounts of undigested residues, using it as a matrix for hormone analysis requires bigger/less diluted samples than recommended for other species.
- The hour of sample collection did not affect hormone levels. Therefore, the fieldwork can be conducted during the day, not only in the early morning, as often suggested.
- To explain the variation in the stress level in otters, more samples, and probably also additional explanatory factors are needed.

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