Stress Protein Expression: An Early Warning Sign of Freshwater Community Degradation via Road Salt Runoff in New Hampshire

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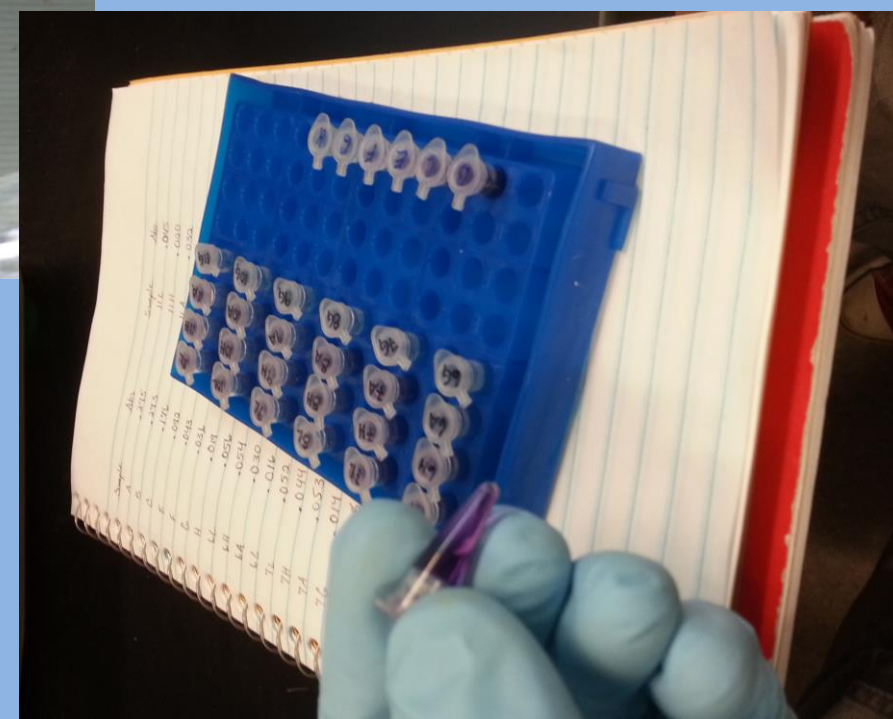
Fig. 1 – Maccaffertium (Flat-headed mayfly)

Fig. 2 – Measuring protein concentrations of samples using BCA assay
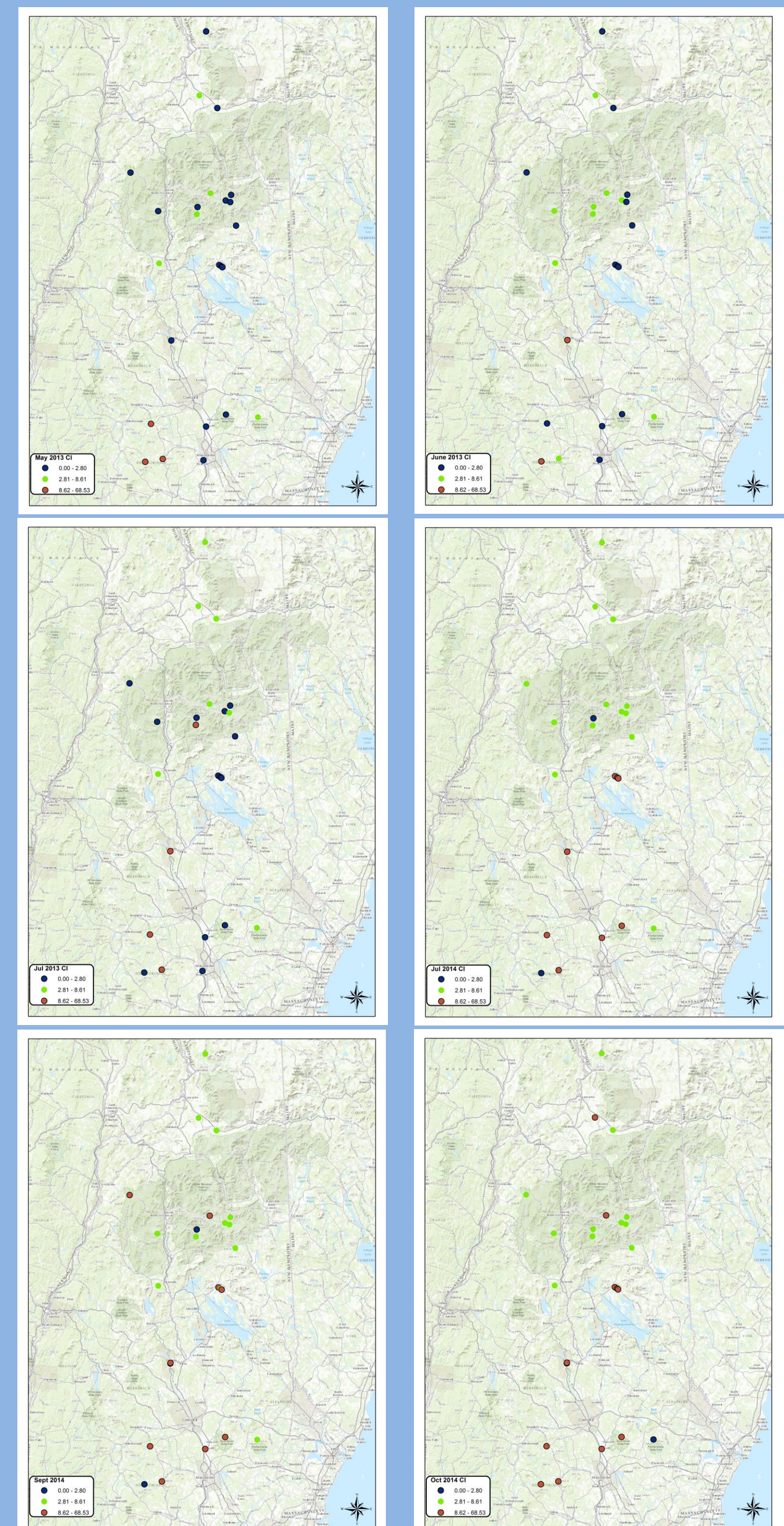

Fig. 5 – Monthly snapshot Chloride (Cl) concentrations at proposed sites selection for Summer 2016 (data from LoVoTECs lovotecs.blogspot.com/2015_05_01_archive.html)

Background

The widespread application of chloride salt control products for winter travel safety has caused growing concerns over their impact to nearby aquatic ecosystems. In order to identify ecosystems affected by runoff of road salts, we hope to establish a simple, fast process for early identification of at-risk communities, essentially providing time for adjustment of road maintenance practices before the loss of sensitive species. We will be testing a new method of using mayflies as bioindicators of stream health with molecular techniques to examine the expression of heat shock proteins in mayfly nymphs.

- From 2008-2010, the number of documented streams in NH impacted by road salt runoff jumped from 19 to 40 (NH DES, 2014).
- Salinization of freshwaters has been observed to vastly alter benthic macroinvertebrate assemblages, particularly decreasing the variety of mayflies present (Doupe & Horwitz, 1995).
- Mayflies represent 25-50% of riffle taxa in some regions (Pond, 2010).
- Heat shock protein (HSP) are a class of molecular chaperones whose purpose is the protection and maintenance of other proteins in response to emergency stress (Lencioni et al. 2009; Hochachka & Somero, 2014)
- HSPs actively observed in natural populations in response to occasional stress exposures (Sørensen et al. 2003)

While a multitude of research has focused on the lethal dosages of NaCl exposure in mayfly nymphs, little has been accomplished in establishing sub-lethal indicators of osmotic stress, which may serve as a more sensitive and effective method for determining biotic responses to disturbance (Sørensen et al. 2003; Johnson et al. 2015).

Objectives

I. Establish the location and degree of HSP expression throughout the mayfly morphology
II. Develop standard curve describing the expression of HSP's in mayflies as a function of salt loading
III. Examine levels of HSP expression in mayflies across a gradient of salt concentrations in NH streams

Current & Planned Research

1) Analysis of HSP70 expression across mayfly morphology in *Maccaffertium* nymphs (Fig.1)
- Mayfly nymphs have been dissected into four body regions: Head, Gills, Legs and Abdomen.
- Total protein content has been extracted using T-PER lysis buffer and quantified using a standard Bicinchonic Acid Assay (BCA) + nanodrop (Fig. 2)
- Total protein extracts from each region have been equalized and will be examined for differential expression of HSP70 by Western-Blotting (Fig. 3&4)

2) Generation of mayfly salt-response curves
- Site Selection – 10 Stream sampling sites will be determined through examination of water chemistry across the state using the PSU LoVoTECS network and fish sampling data from NH Fish & Game (Fig. 5)
- Mesocosm aquaria mimicking stream environments will be used as housing in an attempt to limit handling stress (see Fig. 6)
- Following three day acclimation period, nymphs will be exposed to a variety of salt concentrations
- Total protein contents will be extracted and examined for levels of HSP70 expression corresponding to severity of salt treatment

3) Quantification of in-situ HSP70 expression in NH streams
- Specimen collection will occur once per month May-September with a goal of no less than 30 specimens per site, per month
- To avoid any bias of stress-induction through handling/travel, individuals will be flash frozen in liquid nitrogen immediately following collection in the field
- Each frozen specimen will undergo protein extraction identical to the previous experiments, expression levels of HSP70 will be compared to previously generated salt-stress response curves to examine correlations between habitats
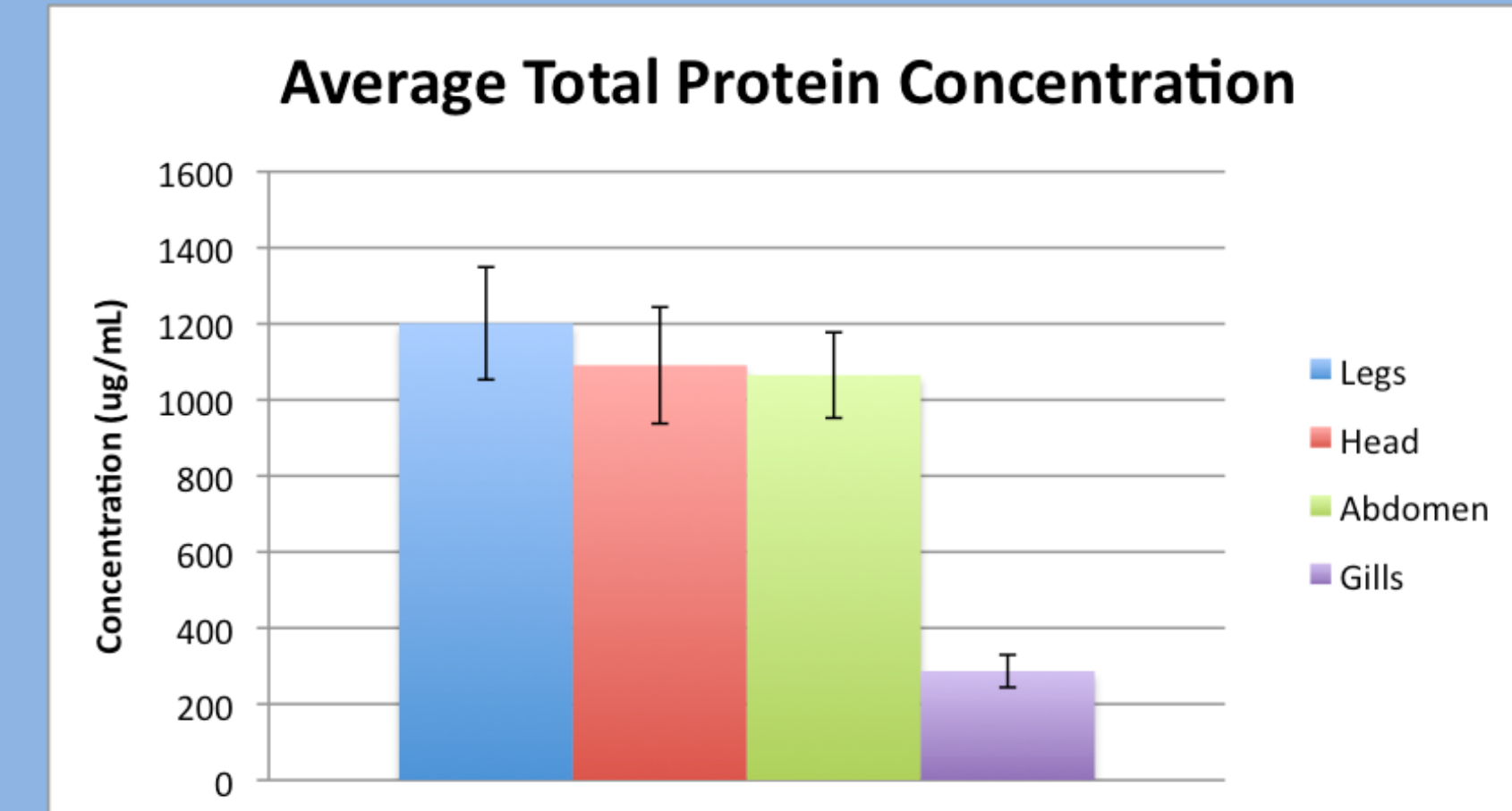

Average Total Protein Concentration
Fig.3 – Total Protein concentrations of Glove Hollow samples

Legend: Legs, Head, Abdomen, Gills

Fig. 4 – Incubation of Western Blot
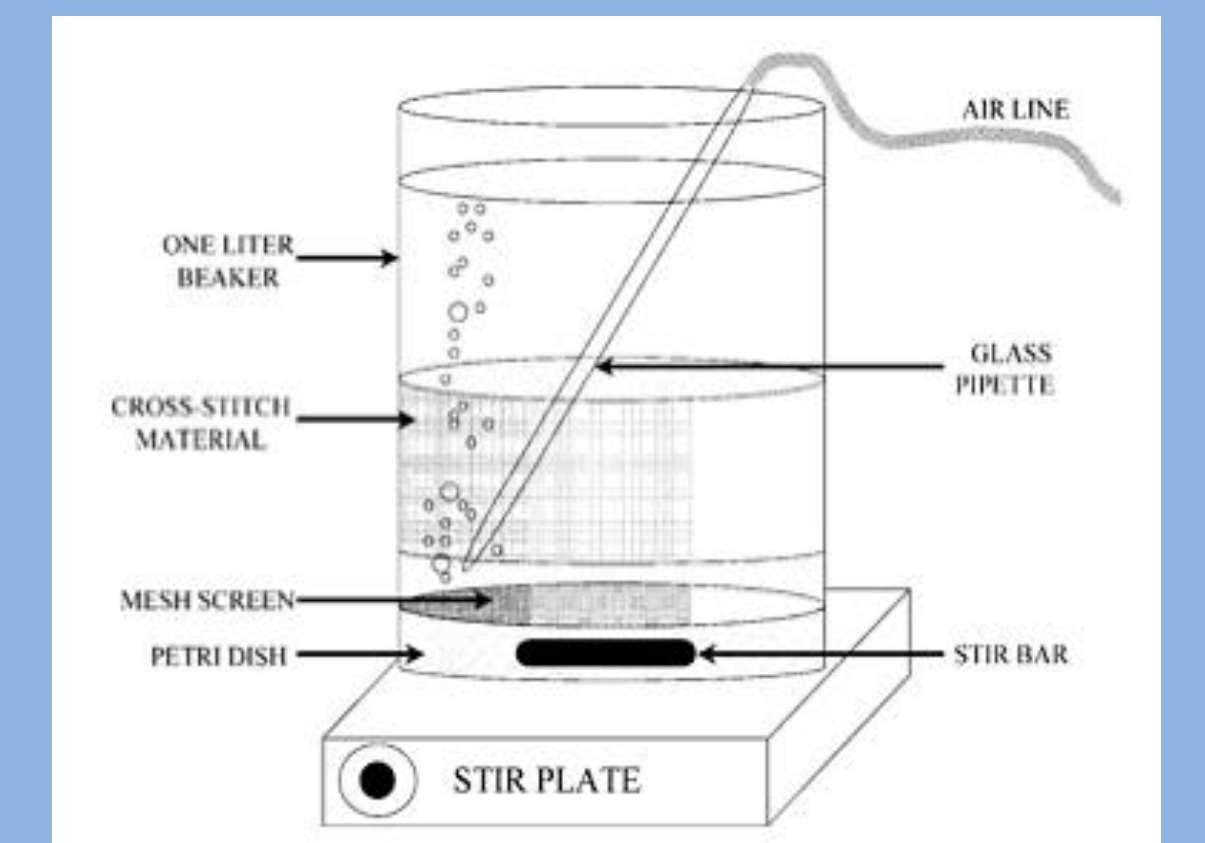
Fig. 6 - Laboratory setup of Kennedy et al. (2004)

Literature Cited
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