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NOSTOC (CYANOPHYTA) PRODUCTIVITY IN OREGON STREAM ECOSYSTEMS: INVERTEBRATE INFLUENCES AND DIFFERENCES BETWEEN MORPHOLOGICAL TYPES[1]

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ABSTRACT

Nostoc parmelioides *colonies housing dipteran larvae (*Cricotopus*) had higher rates of weight specific photosynthesis than colonies without the larvae. A change in colony shape, which allowed the alga to be exposed to higher light intensities, occurred in the presence of the larvae. This change in morphology together with potential nutrient additions by the larvae and other effects may have caused the increase in photosynthetic rates.* Nostoc *colonies were typically found in open areas of small streams in western Oregon mountains where the ability to respond to high light would be advantageous in supporting the metabolically expensive process of nitrogen fixation.*

Key index words: Algal-invertebrate interactions; blue-green algae; chironomid; Cricotopus nostocicola; Nostoc parmelioides; *stream algal productivity*

Several groups of algae exhibit different morphological stages in their life cycle. Of the macroalgae, the heteromorphic mode of existence is most frequently found in the Chlorophyta, Phaeophyta, and Rhodophyta (Lubchenco and Cubit 1980). However, also members of the Cyanophyta (cyanobacteria), particularly species of *Nostoc,* are polymorphic (Lazaroff 1973). The ecological advantages of these different morphological types have not always been clear, although in marine macroalgae it has been suggested as an adaptation to fluctuations in grazing pressure (Lubchenco and Cubit 1980).

An unusual mutualistic relationship and perhaps a rare example of co-evolution among stream plants and animals (Gregory 1983), exists between two species of chironomid midges, *Cricotopus* (*nostocicola* and *fuscata*) (Diptera: Chironomidae), and a nitrogen-fixing, blue-green alga, *Nostoc parmelioides,* in western mountain streams (Wirth 1957, Brock 1960). During the early summer, females lay eggs on the surface of developing *Nostoc* colonies. After hatching, larvulae bore into the colony and eventually a single larva is housed, until emergence of the adult, within the colony. The midges appear host-specific; that is, they have not been observed in the free-living state or in other plants, and by later instars, do not move from colony to colony. Upon entry of the midge, the *Nostoc* colonies which house the midges change in shape from globose, essentially spherical macrocolonies to flattened, more erect forms resembling discs or small "ears." The *Nostoc* colony resumes its globose form and decomposes after emergence of the adult midge.

Both *Nostoc* ears and unoccupied spheres are present throughout the year in Oregon streams which have abundant *Nostoc parmelioides,* although ears are more prevalent in summer and spheres in winter (Ward, unpublished data). This relationship allows the larvae to grow in a protected environment with a readily available, high nitrogen food source within the colony. The advantages to the algal colonies, which are being consumed from the inside out, have been less obvious, although the larva may on occasion aid in attaching the colony to the substrate. We present evidence that the ear-shaped *Nostoc* colonies with the *Cricotopus* midge have much higher photosynthetic rates year round than the globose, spherical colonies and suggest that the midge provides the stimulus for the change in colony shape, which together with the presence of the midge, allows the alga to exist in and respond to a high light regime.

MATERIALS AND METHODS

Seasonal and diel measurements of *Nostoc* primary productivity were made in "*Nostoc* Creek," a second order mountain stream located in the H. J. Andrews Experimental Forest in the Cascade mountains of central Oregon. This creek is an unnamed tributary of Lookout Creek on U.S. Forest Service maps. One bank of the stream is adjacent to an oldgrowth of Douglas fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) forest; whereas, the other side abutts an experimentally logged and subsequently thinned section with fewer trees. Because of the thinning, parts of the stream surface are unshaded and receive full sunlight from about 11:00 to 15:00 h with some seasonal variation. The stream is shallow (<0.25 m) and has a rocky substrate to which the *Nostoc* colonies attach (Fig. 1).

Primary productivity measurements were made by incubating rocks with attached *Nostoc* colonies in approximately 1.5 L Plexiglas® recirculating chambers (modified from Bott et al. 1978). Rocks were placed in the chamber with streamwater, injected with 4.0 mL $NaH^{14}CO_3$ (5.12 μCi/mL), sealed, and set in the stream near the site of rock collection. After 3 to 4 h, the rocks were removed, rinsed with nonradioactive stream water, placed in plastic Zip-loc® bags and returned to the laboratory in the dark in an ice chest. At the laboratory, the *Nostoc* colonies were removed from the rocks, the two types of colonies were separated, dried to constant weight and stored in a desiccator. Preliminary

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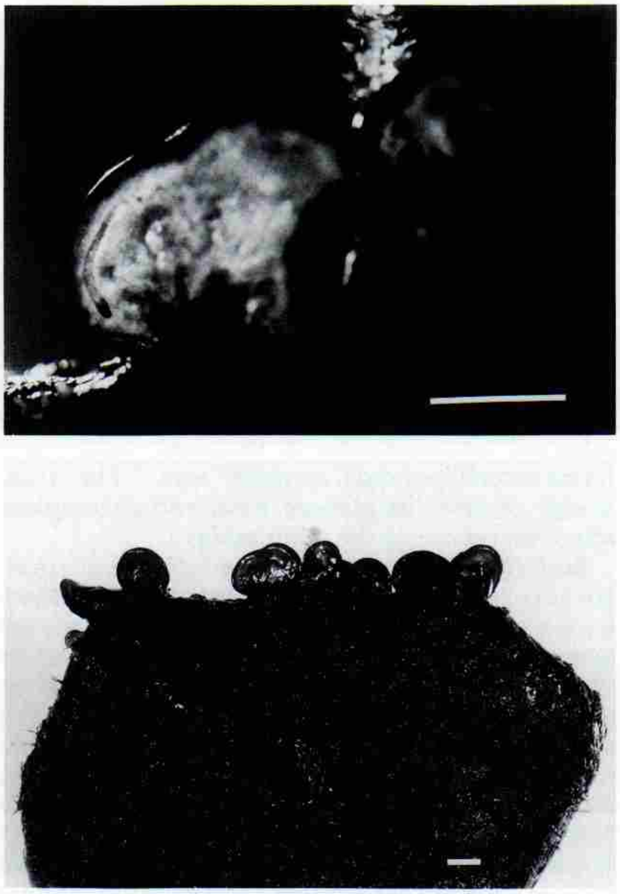

FIG. 1 (Upper): Backlighted *Nostoc* "ear" containing *Cricotopus* midge in left side of colony. Bar = 5 mm. (Lower): *Nostoc* "ears" attached to rock. Bar = 5 mm.

experiments, in which weights of midges versus *Nostoc* colonies were compared, indicated that midges made up a small portion (<10%) of the entire colony, therefore, midges were not removed before analysis. If the midges had been removed, the difference between ear and sphere weight specific photosynthesis would have been even greater than reported here since weight would have been deleted from the ears and not the spheres. The dried material was weighed and combusted to CO_2, which was trapped in a CO_2-trapping scintillation cocktail in a Packard sample oxidizer (Model 306). Samples were radioassayed in a Beckman LS 7500 scintillation counter. Values were corrected for oxidation efficiency (always greater than 99%) and quenching.

Nutrient samples were analyzed on a Technicon II Autoanalyzer. Temperature measurements were made with a YSI thermistor (Model 43). Alkalinity was measured by titrating a known volume of streamwater with 0.02 N H_2SO_4 to pH 4.8 and pH was measured with a Coleman pH meter (Model 37A). A Li-Cor (Model 550) printing integrator was interfaced with a pyranometer light sensor to estimate solar irradiance to the stream surface. The light sensor was placed adjacent to the recirculating chambers during incubation.

Nutrient bioassays were performed by incubating triplicate *Nostoc* ears and duplicate spheres in 60 mL serum bottles sealed with rubber stoppers to which 30 mL of streamwater and 0.5 mL $NaH^{14}CO_3$ (see above) were added. Bottles were placed in Nostoc Creek near the site of collection for about 6 h. The *Nostoc* material was removed from the bottles at the end of the incubation period and analyzed as described above. Nutrient additions to duplicate treatment bottles were injected to yield a final added concentration in μg/L of: 100, NH_4-N (added as NH_4Cl), 50, PO_4-P (added as $K_2HPO_4 \cdot 3H_2O$); 5, Mo (added as $Na_2MoO_4 \cdot 2H_2O$); and 5, Fe (added as $FeCl_3$). Streamwater contained 35 μg PO_4-P$\cdot L^{-1}$, and 5 μg NH_4-N$\cdot L^{-1}$. NO_3-N concentrations were below the limit of detection (<2 μg/L).

RESULTS AND DISCUSSION

Seasonal changes in rates of primary production in shaded and open areas of Nostoc Creek indicated consistantly higher rates of photosynthesis/algal dry weight associated with *Nostoc* colonies containing the chironomid midge compared to colonies without the midge (Fig. 2). These increases were most apparent in the open area which received maximum radiation with maximum photosynthetic rates of ears greater than six times that of *Nostoc* spheres incubated in the same chamber (August 1981). In shaded areas, photosynthetic rates of ears were maximally three times that of spheres in September 1981 and April 1982. Photosynthetic rates of *Nostoc* apparently responded more to changes in irradiance than temperature since photosynthetic rates remained high on sunny days in winter months (e.g. December 1981). Rates of photosynthesis depicted in this figure represent the physiological responses of the algae and not estimates of total carbon input seasonally to the stream reach, since the values were not corrected for total biomass/stream area. Algal biomass in many Cascade mountain streams decreases in the winter months because of heavy precipitation and subsequent scouring and rearrangement of rocky substrates (Rounick and Gregory 1981). However, those *Nostoc* colonies that remain, appear photosynthetically adapted to a wide temperature range (7–15° C).

Comparison of photosynthetic rates of ears versus spheres over a 24 h period on a sunny day in August (1980) further demonstrated that colonies with midges were much better able to respond to midday high light intensities than spheres (Fig. 3). Photosynthetic rates of *Nostoc* ears much more closely paralleled incoming solar irradiance than those of the spheres. Light and photosynthetic data for *Nostoc* ears and spheres from Figure 3 were subjected to linear regression. The r^2 value for ears was 0.98 compared to 0.45 for spheres. This response may be due to a decrease in self-shading associated with a disc-shaped, flattened colony compared to a spherical colony. For both ears and spheres, maximum photosynthetic rates occurred between 14:00 and 17:00 unlike the mid-afternoon photosynthetic depression, typically reported for other aquatic plant communities (e.g. phytoplankton and aquatic macrophytes) and attributed to photorespiration (Hough 1974, Wetzel 1983).

The exact metabolic cues for the change in *Nostoc* shape and the physiological differences are unknown, but we suggest that the potential effects of the midge on the *Nostoc* colonies may include some

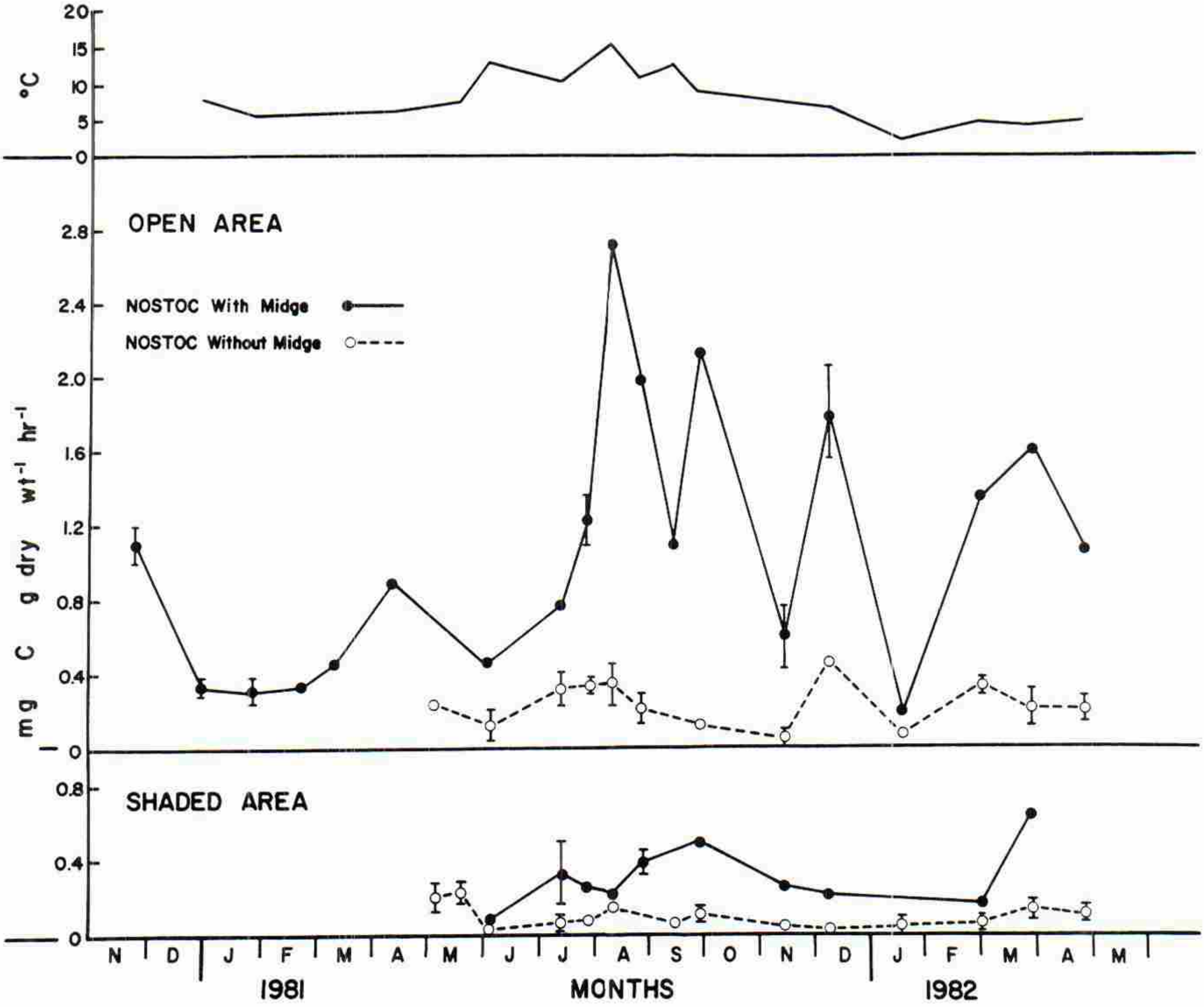

FIG. 2. Seasonal changes in temperature and in rates of primary production of *Nostoc* colonies with and without the *Cricotopus* midge in open and shaded areas of *Nostoc* Creek. Bar = 2 standard deviations.

or all of the following. First, the *Cricotopus* midge may cause the algal colony to change from a globose form to a flattened, thinner, more erect form with a higher surface area to volume ratio. This form, which extends away from the rock, is exposed to higher light intensities than the spheres which are more closely appressed to the rock surface. The flattened shape would decrease the distance from the surface of the colony to the interior, which together with altered flow patterns around the thallus, may facilitate nutrient (particularly CO_2) transport. Second, midge respiration could supply an endogenous source of CO_2, increasing levels of free CO_2 within the colony and augmenting CO_2 for carbon fixation. Paerl (1983) has shown that internal cells of *Microcystis* colonies can become CO_2 limited. Third, the midge may release other inorganic or organic nutrients which stimulate photosynthetic rates. Our initial experiments with nutrient addition bioassays (ammonium-nitrogen, molybdate, iron, and orthophosphate) did not significantly (at 5% level, one-way ANOVA) increase rates of photosynthesis over the control in either ears or spheres. There may be combinations of other inorganic or organic nutrients not tested which may cause a response, or longer experiments may be necessary. Fourth, grazing by the midge may remove the senescent or dead algal material within the colony thereby stimulating apparent photosynthetic rates per unit algal weight. Our data initially favor mechanism one with some support for mechanisms two and four. Additional experimental work with nutrient additions are needed before eliminating mechanism three. The effects of the midge on *Nostoc* photosynthesis suggested here are in agreement with earlier reports on invertebrate effects on algae (Brock et al. 1969, Hargrave

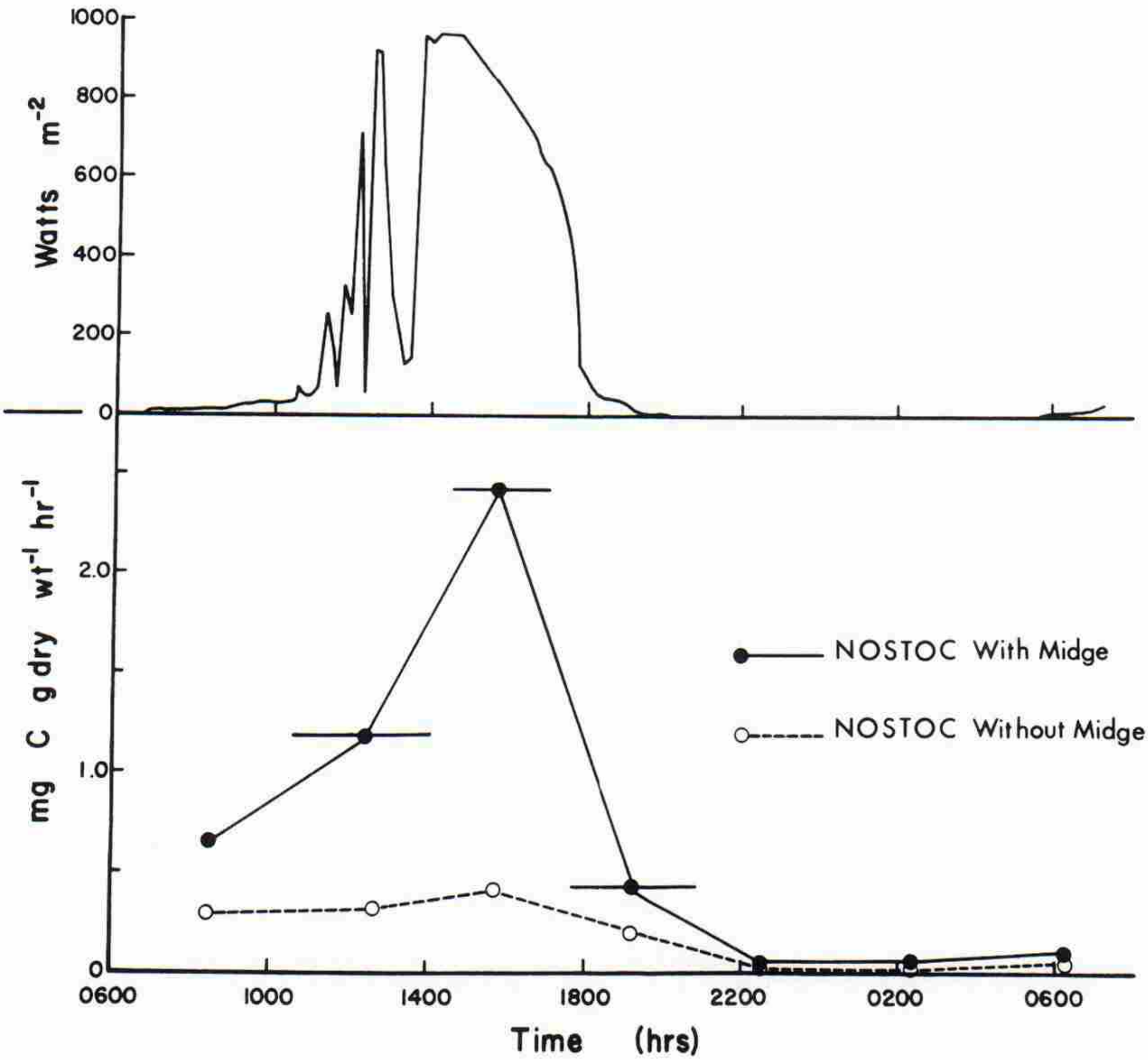

FIG. 3. Diel changes in rates of photosynthesis of *Nostoc* colonies with and without the *Cricotopus* midge compared to changes in solar radiation on 14 August 1980. Horizontal bars indicate period of incubation.

1970, Cooper 1973, Flint and Goldman 1975, Porter 1975, 1976, Brenner et al. 1976, Brock 1967, Shapiro 1979, Lynch 1980, Crumpton and Wetzel 1982, Lamberti and Resh 1983).

General characteristics of *Nostoc* and chironomids make these organisms particularly adaptable to the scenario described here. The success of the relationship suggests several requirements for both organisms. For example, bacteria frequently exhibit pleomorphism (plasticity in form) in response to changing environmental conditions and blue-green algae (cyanobacteria) are physiologically and structurally similar to bacteria. *Nostoc* species in general also exhibit polymorphism or formation of different morphological stages in their life cycles (Lazaroff 1973). Other blue-green algal forms (*Aphanizomenon flos-aquae*) have been shown to respond to the presence of an invertebrate (*Daphnia pulex*) by changing colony shape in planktonic situations (Lynch 1980). Therefore, members of this group are genetically predisposed to respond to environmental variations by changing morphology. Not all animals do as well consuming blue-green algal material as the *Cricotopus* midge discussed here. Studies addressing ingestion and assimilation of blue-green algae by aquatic invertebrate grazers produce no clear pattern of suitability of these algae as a food source in lentic or lotic systems, although they are frequently regarded as toxic or unpalatable (reviewed by Porter and Orcutt 1980, Gregory 1983). In our study, gut analyses of *Cricotopus* revealed numerous remains of lysed vegetative cells and intact heterocysts. The midge was feeding directly on the trichomes with no apparent toxic effect, and had little difficulty in assimilating the blue-green algal material. Other studies have indicated that chironomids can ingest and assimilate blue-green algae easily (Izvekova 1971, Kehde and Wilhm 1972). Possibly some chironomids have special digestive enzymes or gut flora with enzymes enabling rapid digestion of the blue-green algal material.

Besides the physiological implications for both the midge and the alga, the results of the *Nostoc-Cricotopus* association suggest important ecosystem modifications with regard to nutrient inputs, apparently linked to the ability of the *Nostoc* colony to respond to and utilize high light intensities. All of the factors influencing distribution of *Nostoc* ears in Oregon streams are not clearly understood, but there is a strong, positive correlation between *Nostoc* distribution and open-canopied first or second-order streams which have been clear-cut, or streams in second growth watersheds (<35 years since clear-cutting) with an alder-dominated riparian zone (Ward 1985). These ecosystems are characteristically nitrogen-limited with high concentrations of both inorganic and organic phosphorus (PO_4-P > 35 μg/L; organic P = 26–80 μg/L). The *Nostoc* ears function in supplying these streams with nitrogen, made possible by the metabolically expensive and high energy requiring process of nitrogen fixation. The presence of the *Cricotopus* midge apparently triggers a change in morphology which appears to enhance the ability of the *Nostoc* colony to respond to the metabolic requirements of this process. Linkages between the *Cricotopus* life cycle (e.g. site and timing of egg deposition) and *Nostoc* ear distribution, and the exact metabolic cue for the proposed midge-induced change in *Nostoc* shape, are areas which need further investigation.

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