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NOTE

Nitrogen-fixation by the cyanobacterial symbiont of the diatom genus *Hemiaulus*

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ABSTRACT: Nitrogen-fixation by cyanobacterial symbionts in the oceanic diatoms *Hemiaulus membranaceus, H. hauckii,* and *H. sinensis* was documented in the Southwestern Atlantic Ocean. All *Hemiaulus* cells with diatom chlorophyll autofluorescence contained fluorescent symbionts undetectable by standard light microscopy. Average cell-specific ethylene reduction rates (2.3 to 5.3 $\times$ 10^{-13} M ethylene cell h^{-1}) were 2.2 to 5.2 times lower than calculated rates from *Rhizosolenia-Richelia* blooms in the central Pacific gyre and 26 times lower than results from *Richelia*-containing *Rhizosolenia* cultures. Calculations suggest that the N_2 fixation associated with *Hemiaulus* is 21 to 45 times greater than that from the *Rhizosolenia-Richelia* association in the southwestern Atlantic Ocean.

Hemiaulus is a common and ubiquitous diatom genus typically dominating or co-dominating the diatom community after seasonal stratification in warm oligotrophic seas (Guillard & Kilham 1977). A puzzling aspect of its ecology is its persistence over large areas dominated by nano- and pico-plankton despite low ambient nutrients. Periodic blooms of this genus occur in the central Pacific gyre and have been associated with the nitrogen-fixing *Rhizosolenia-Richelia* symbiosis (Venrick 1974). Cyanobacterial symbionts usually identified as *Richelia intracellularis* Schmidt are also known in *Hemiaulus*; however, the identity of the symbiont is uncertain (Sundström 1984). The presence of a heterocyst suggests N_2-fixation is likely in this symbiosis (Kimor et al. 1978, Heinbokel 1986), but N_2-fixation by *Hemiaulus* symbionts has never been examined.

Although the host is frequently seen, the symbiont is rarely reported. Kimor et al. (1978) noted that up to 62 % of the *Hemiaulus membranaceus* Cleve collected off southern California contained symbionts, although more extensive collections offshore contained only 16.1 to 18.6 % symbiotic *Hemiaulus*. Heinbokel (1986) noted that the *Hemiaulus* symbionts were frequently visible only under epifluorescent illumination, and that ca 80 % (n = 668) of the *Hemiaulus hauckii* and *H. membranaceus* in samples north of Hawaii contained symbionts. He suggested that symbiont abundance, and their role in oceanic nitrogen fixation, may have been underestimated due to the need to use epifluorescence to reliably identify the symbiont. In this report, I document N_2-fixation by the *Hemiaulus* symbioses, and present some additional observations on the cryptic nature of the symbiont.

Methods and materials. *Hemiaulus* cells were isolated by micropipet from surface tows (30 cm net, 20 µm mesh) north of St. Johns, U.S. Virgin Islands (13 Feb 1991; 18° 36.43′ N, 65° 27.49′ W), and north of Haiti (15 Feb 1991; 20° 40.15′ N, 72° 54.43′ W). *Hemiaulus* cells (130 to 200 each) were placed into 2 ml screwcap vials equipped with a Teflon lined septum and containing 1 ml of 0.45 µm filtered seawater. Care was taken to exclude trichomes of *Trichodesmium* and cells of *Richelia*-containing *Rhizosolenia* from the vials. Curved chains of *H. hauckii* were present, but were not examined for acetylene reduction due to the difficulty in picking the chains with the micropipet. A blank of filtered seawater was run concurrently. Vials were incubated at 26 °C and 306 µE m^{-2} s^{-1}. Two experiments were run with each net tow collection

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and consisted of one vial of *H. membranaceus* and one vial of combined *H. hauckii* and *H. sinensis*. Acetylene was injected (0.2 ml) into the headspace and subsequent ethylene generation was measured on a Shimadzu Mini 2 gas chromatograph. Ethylene concentrations were corrected for liquid-phase solubility, and successive time series points were corrected for the sample volume withdrawn in the previous assays. The small headspace volume (1 ml) precluded replication of time series samples. Rates of ethylene evolution were determined from the slope of the time-series data.

Symbiont enumeration was performed on a Zeiss Axiomat microscope equipped with epifluorescence capabilities using freshly collected samples. Approximately 10 to 100 cells of each species (depending on its abundance) were examined from the same net tow as the acetylene reduction experiments.

Results and conclusions. Ethylene evolution occurred in all experimental vials containing *Hemiaulus*. Rates of ethylene evolution were linear for at least 4 h (Fig. 1). *H. membranaceus*-containing vials evolved ethylene at rates of 2.3 and 3.6×10^{-13} mol ethylene $cell^{-1}$ h^{-1}, and *H. hauckii-H. sinensis* evolved ethylene at 3.0 and 5.3×10^{-13} mol ethylene $cell^{-1}$ h^{-1}. No symbionts were visible under brightfield or Nomarski illumination; however, under epifluorescence, all *Hemiaulus* spp. except the curved morph of *H. hauckii* contained 1 or 2 symbionts (Fig. 2). The curved *H. hauckii* (n = 30) showed no host chlorophyll autofluorescence, contained no symbionts, and appeared dead. These observations are similar to Heinbokel's (1986) observations of ca 80% symbiont-containing *Hemiaulus* cells north of Hawaii. Although *H. hauckii* and *H. sinensis* were not examined individually for acetylene reduction, it is reasonable to assume the symbionts of both species were fixing nitrogen. Average host cell-specific rates of ethylene evolution for the

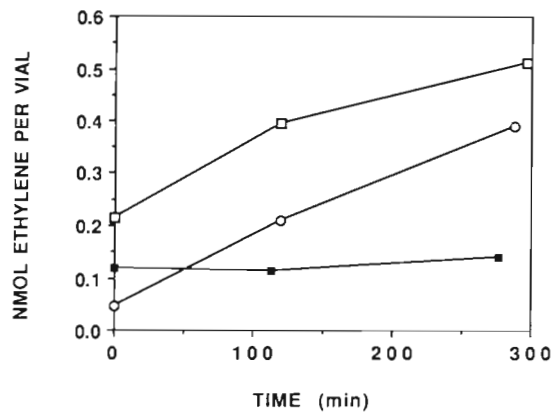

Fig. 1 Time course of ethylene evolution from samples collected on 13 Feb. 1991 (□) *Hemiaulus membranaceus*; (□) *H. hauckii-H. sinensis*; (■) blank values

Hemiaulus symbioses (3.6×10^{-13} mol ethylene $cell^{-1}$ h^{-1}) were 2.2 to 5.3 times lower than field rates of ethylene evolution (0.8 to 1.9×10^{-12} M ethylene trichome h^{-1}) calculated by Villareal (1990) for field data of Mague et al. (1974) on *Rhizosolenia-Richelia*, and were ca 26 times lower than ethylene evolution rates (9.5×10^{-12} M ethylene trichome h^{-1}) from laboratory cultures of the *Rhizosolenia-Richelia* symbiosis at 300 μE m^{-2} s^{-1} (Villareal 1990).

The nitrogen fixation by these often cryptic *Hemiaulus* symbionts is an undocumented new production source. The significance of the nitrogen input can be estimated for the summer central North Pacific gyre where a conservative calculation based on the minimum acetylene reduction rate, a 4:1 acetylene reduction-N_2 fixation ratio, and a 30 m vertical N_2 fixation zone, suggests that at a typical abundance of 100 $cells^{-1}$ (Venrick unpubl. obs.), *Hemiaulus* could contribute 4.8 μg-at. N m^{-2} d^{-1} or ca 15% of the average N_2 fixation (33 μg-at. N m^{-2} d^{-1}; Mague et al. 1974). Blooms of 25000 *Hemiaulus* cells l^{-1} are reported in this region (Venrick 1974), and could have an important effect on nutrient dynamics in the region if symbiont nitrogen fixation occurs at similar rates.

It is difficult to address further the quantitative importance symbiotic N_2 fixation. *Hemiaulus hauckii* is capable of up to 3.8 divisions d^{-1} at 24°C in laboratory culture (Brand & Guillard 1981) while *H. sinensis* may grow at up to 1.99 divisions d^{-1} at 25°C (Brand et al. 1983). Field growth rates of 4.3 divisions d^{-1} for *H. hauckii* and 2.2 divisions d^{-1} for *Hemiaulus* spp. have been reported from cage cultures off the west coast of Florida (Vargo 1983) and off the Great Barrier Reef (Furnas 1991), respectively. Like symbiotic *Rhizosolenia* (Villareal 1990), *Hemiaulus* probably obtains fixed nitrogen from the symbiont that serves to sustain the host diatom under oligotrophic conditions. Should the symbiont be capable of sustaining these host high growth rates, then explosive inputs of new nitrogen via symbiotic nitrogen-fixation may be occurring in oligotrophic tropical and subtropical seas.

The importance of this question is apparent from the tropical-subtropical distribution of this genus. After seasonal stratification, *Hemiaulus* can represent one of the dominant diatom taxa in the Sargasso Sea (Hulburt et al. 1960, Hulburt 1966), the Aegean Sea (Ignatiades 1969) and the central Pacific gyre (Venrick 1969). One or 2 *Hemiaulus* species frequently dominated the diatom flora in the Northwest Providence Channel, Bahamas from 1964 to 1966 (Wood 1968), and it is 'frequent and abundant' (up to 153 300 cells l^{-1} of *H. sinensis*) on the west coast of Florida (Saunders & Glen 1969.). Near-shore and far-shore average summer abundance of *H. sinensis* is 3924 and 5668 cells l^{-1}, respectively, in the Mid-Atlantic Bight region of the

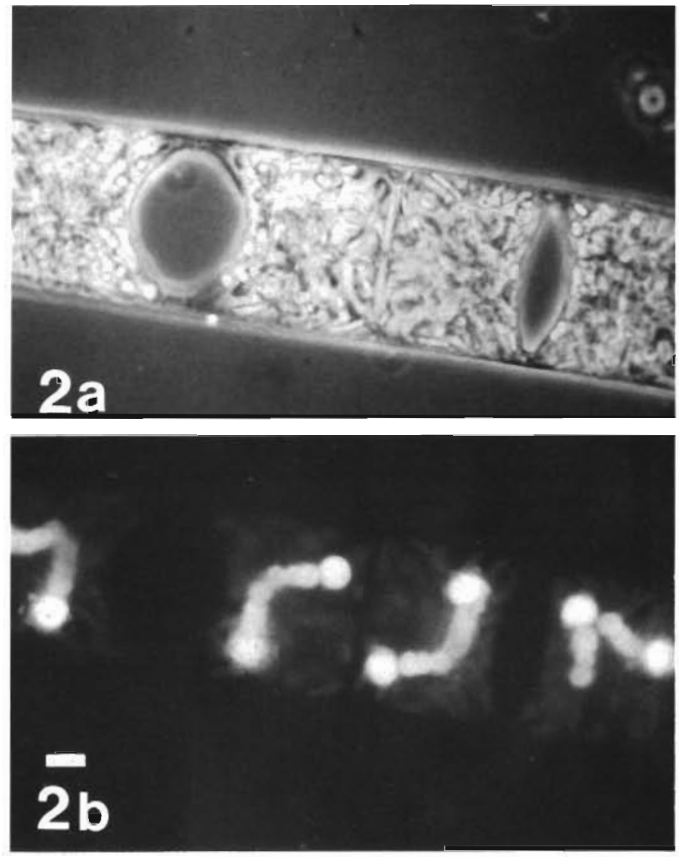

Fig. 2. *Hemiaulus membranaceus* chain. (a) Phase contrast illumination. Note that the symbionts are not visible. (b) Epifluorescent illumination. Note that each cell has 2 symbionts. Scale bar = 8 μm. Photographs courtesy of D. Caron

eastern USA (Marshall & Cohn 1987). It is evident that *Hemiaulus* is an important and often abundant component of the diatom community.

The distribution of symbionts is more problematic. *Hemiaulus* spp. are far more abundant than the commonly noted *Rhizosolenia-Richelia* symbiosis (Gilmartin & Revelante 1974, Venrick 1971, 1974). For example, *Hemiaulus* occurred at 15 to 140 cells l^{-1} in the southern and central Sargasso Sea, while *Rhizosolenia-Richelia* was not found (Hulburt et al. 1960, Hulburt 1962). Hargraves et al. (1970) noted that *Richelia* was present at only 2 of 17 stations in the Lesser Antilles region, but *Hemiaulus* was present at 13 of 17 stations. The reported symbiosis percentages of *Hemiaulus* in Heinbokel (1986) and in this study (80 to 100 %) suggest that symbionts could be present in a large proportion of these cells. In the net collections from this study, only 3 *Rhizosolenia-Richelia* associations were seen, while over 300 symbiotic *Hemiaulus* spp. were observed. Based on this relative abundance, the measured field rates of acetylene reduction by their symbionts, and assuming a constant conversion ratio of acetylene reduction : N_2 fixation, it appears that 21 to 45 times as much symbiotic N_2 fixation was occurring by *Hemiaulus* associations as by *Rhizosolenia* associations at the Caribbean study sites.

Current N_2-fixation surveys may be missing a substantial fraction of total nitrogen fixation by overlooking these cryptic symbioses. A major unresolved question is whether all *Hemiaulus* contain symbionts, and the degree to which these symbionts are present in, and contribute to, *Hemiaulus* bloom formation or maintenance. As a final note, other diatoms are noted to contain coccoid cyanobacterial symbionts that may be capable of N_2 fixation (Norris 1967, Hallegraeff & Jeffery 1984). These symbioses also require further study to examine their contribution to N_2 fixation.

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