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## An experimental approach to understand trophic interaction of photosymbiosis in planktic foraminifers

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Planktic foraminifers are marine heterotrophic protists. Of about 50 species of modern planktic foraminifers, about 10 species that especially dominate in warm and low-nutrient surface water harbor autotrophic algae as endosymbionts (photosymbiosis). It is generally considered that foraminifers benefit from photosynthates of symbionts, and in return, they provide nutritious environment for symbionts to live. At the same time, however, the host's degree of dependence on symbionts is still enigmatic. This is because growth of the host primarily depends on food (prey) availability. In this context, a common assumption that photosymbiosis is an advantageous ecology for host foraminifers to live in oligotrophic oceans still has room to discuss. To understand trophic interaction between host and symbionts, we conducted culture experiments and analyzed vitality of host-symbiont consortia under controlled nutrient conditions.

We cultured dinoflagellate-bearing species *Globigerinoides sacculifer* for two weeks. Assuming the two sources of nutrients for symbionts, i.e., from the host's metabolites and from the ambient seawater, we controlled feeding regime (fed Artemia every other day or unfed) and nutrient concentration of culture media (0.22- $\mu$ m filtered seawater [SW] or nutrients-added filtered seawater [NSW]). Four experimental groups are set; (a) fed and SW, (b) unfed and SW, (c) fed and NSW, and (d) unfed and NSW. Nutrient concentrations of SW and NSW were respectively 0.2 and 16  $\mu$ mol L<sup>-1</sup> of NO<sub>3</sub>+NO<sub>2</sub>, and 0.07 and 1.0  $\mu$ mol L<sup>-1</sup> of PO<sub>4</sub>. Temperature was set to 26.5-27.5 °C. Photosynthetic active radiation was set to 170-220  $\mu$ mol quanta m<sup>-2</sup> s<sup>-1</sup>, and its light/dark cycle was 14/10 hours. Test growth of the host, chlorophyll content, and photo-physiology of the symbionts were used as criteria of their vitality. We measured maximum test length of host foraminifers and chlorophyll fluorescence of individual host-symbiont consortium during the culture period almost every day. For fluorometric analysis, we used fast repetition rate (FRR) fluorometry. From FRR measurement,  $F_m$  (an index of chlorophyll content), and  $F_v/F_m$  (an index of potential photosynthetic activity) were obtained and analyzed for each individual consortium.

During the culture period, foraminifers grew and formed new chambers in the fed groups (a, c). On the contrary, specimens in the unfed groups (b, d) gradually decreased their cytoplasm volume, and in accordance with the decrease they often shed chambers one by one. The chlorophyll content, thus the biomass of symbionts per foraminifer, tended to increase in the fed groups (a, c), whereas it decreased or kept nearly constant in the unfed groups (b, d). Despite the apparent diminishment of the unfed groups,  $F_v/F_m$  was significantly higher in the unfed groups (b, d) than that in the fed groups (a, c). It indicates that symbionts in starved foraminifers photosynthesized more actively. Nutrient concentration in the culture media (SW or NSW) did not necessarily affect on  $F_v/F_m$ .

Considering the fact that foraminifers maintained their life and symbionts were capable of photosynthesis in starved condition, it can be said that foraminifers have survived only by photosynthates derived from the symbionts or digesting the symbionts themselves for about two weeks of the culture period. If this relationship is true in natural environment, photosymbiotic interaction should help foraminifers to survive for certain duration even if they cannot capture any prey. This should be an advantage for them to live in low-nutrient and well-lit environment.

Keywords: planktic foraminifers, photosymbiosis, nutrients, FRRF, culture