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# Application of chlorophyll fluorescence technique in the study of coral symbiotic zooxanthellae micro-ecology



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#### ABSTRACT

Mutualistic relationship between coral polyps and their symbiotic zooxanthellae living within their tissues are the most essential features of a coral reef ecosystem. In this symbiotic system, the coral polyps provide a protected habitat, carbon dioxide and nutrients needed for photosynthesis to zooxanthellae; in turn, the symbiotic zooxanthellae provide food as products of photosynthesis to coral polyps. The Photosynthesis of zooxanthellae is therefore an important process of this symbiotic system as well as the development of the whole coral reef ecosystem. The recent application of chlorophyll fluorescence technique in the study of the zooxanthellae's photosynthesis has greatly improved our understanding on the micro-ecology of corals and the symbiotic zooxanthellae. This paper summarizes the recent progress as the following aspects: (1) The ecological characteristics of the photosynthesis of symbiotic zooxanthellae, such as the diurnal and seasonal changes in the photochemical efficiency of the zooxanthellae, and the relationship between zooxanthellae photosynthesis and the world-wide coral bleaching. (2) The mechanism of corals acclimating to the changes of irradiance via spatial and temporal photoacclimations, including the corals' photobiology; zooxanthella size, pigmentation, location and clade, and the relationship between light extremes and the corals' metabolism and calcification. (3) The understanding of the response of zooxanthellae to various environmental stresses, such as long-term changes in the chlorophyll fluorescence of bleached and recovering corals; the tolerance of corals to thermal bleaching; the changes to photosystem II of symbiotic zooxanthellae after heat stress and bleaching. Due to the above findings, the chlorophyll fluorescence values of those coral species sensitive to environmental changes have been utilized as indicators of coral health as well as the status of coral reef ecosystems. In summary, the chlorophyll fluorescence technique has great potential in the understanding, monitoring, protecting and managing coral reefs.

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#### 0. Introduction

This mutualism between *Symbiodinium* spp. and tropical and sub-tropical coral species has been a key component for the reefbuilding corals. These algae are often referred to by the term of "zooxanthellae" commonly. They have chlorophyll a and accessory pigments (e.g. other chlorophylls, carotenoids and phycobilins) for photosynthesis and provide products of photosynthesis to the host of corals. It is estimated that there are about one million symbiotic zooxanthellae cells per square centimeter of coral tissues [1], and up to 90% of the coral metabolic demand from the by-products of photosynthesis by the symbiotic zooxanthellae [2]. Therefore, the

growth of reef-building corals and the status of coral reef ecosystem are closely related to the photosynthesis of zooxanthellae.

Chlorophyll fluorescence technology, which is developed in recent years, is a new measurement and diagnosis method to explore the photosynthesis of symbionts. Due to the fast, sensitive and non-invasive characteristics, this technology has been attracted more attention in the research of zooxanthellae physiology and coral reef ecology. As the improving of measure instrument, some of which is submersible, this technology will be used widely and play an increasingly important role in promoting the solutions for some key ecological problems.

The application of chlorophyll fluorescence technology in the research of coral reef has been summarized from five aspects by Zhou et al. [3], with particular emphasis on the role in macroecology. This paper will focus on the progress of micro-ecology by means of chlorophyll fluorescence technology, and expect to

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promote the comprehensive application of this technology in the fundamental research, monitoring and management of coral reef.

### 1. Ecological characteristics of photosynthesis of symbiotic zooxanthellae

Some fluorescence parameters measured by chlorophyll fluorescence technology revealed the ecological characteristics of photosynthesis of symbiotic zooxanthellae in corals, such as the diurnal and seasonal variation of the zooxanthellae photosynthesis, the spatial and inter-specific differences in the photochemical efficiency, and main driving factors of zooxanthellae photosynthesis.

### 1.1. Diurnal and seasonal variation of the zooxanthellae photosynthesis

Diurnal variation of the photosynthesis existed in the symbiotic zooxanthellae of corals. Jones and Hoegh-Guldberg revealed that dark-adapted Fv/Fm (PS II maximum quantum yield) showed clear diurnal changes, decreasing to a low at solar noon and increasing in the afternoon [4]. The effective quantum yield of photosystem II ( $\Delta F/Fm'$ ) measured under ambient light also displayed obviously diurnal changes [5–7].  $\Delta F/Fm'$  was high when the photosynthetically active radiation (PAR) was low in the morning and evening, and reduced with the increasing of PAR at noon. According to the linear fitting from the relation between  $\Delta F/Fm'$  and PAR,  $\Delta F/Fm'$  rose with the increasing of radiation intensity when it was less than the minimum saturation radiation (Ek), and reduce rapidly while radiation intensity exceed the Ek [7,8].

Seasonal variation of photosynthesis existed in the symbiotic zooxanthellae of corals. In previous seasonal studies, Montastraea spp in the Caribbean [9] and Favia favus and Stylophora pistillata in the Red Sea [10] showed that the zooxanthellae generally had higher dark adapted maximum quantum yield (Fv/Fm) in winter than that in summer. The former study found that Fv/Fm was correlated to both light and temperature, while the latter found that Fv/Fm was correlated only with light not temperature. Seasonal variability in temperature may explain the discrepancy in temperature effects on seasonal Fv/Fm. Seasonal variability in maximum daily water temperatures was 11 °C in the Bahamas [9], and only 6 °C in the Red Sea [10]. Piniak and Brown reported temporal variability in fluorescence parameters measurements for 10 coral species in Ofu, American Samoa [11] which all had higher dark adapted maximum quantum yield (Fv/Fm) and higher lightadapted effective quantum yield ( $\Delta F/Fm'$ ), and lower relative electron transport rates in winter (rETR) than that in summer. It verified that seasonal variation of photosynthetic efficiency was not accidental. It may be common in various types of corals from various geographic locations. Meanwhile, it also confirmed the effect of temperature variation on the seasonal change of photosynthesis. Seasonal differences in fluorescence parameters were usually observed in the habitat with a more variable temperature regime, while differences between species were observed only in the more environmentally stable habitat.

## 1.2. Spatial and inter-specific differences in the photochemical efficiency of zooxanthellae

Symbiotic zooxanthellae of corals grew up in different depth had obvious differences in photochemical efficiency. Winters et al. performed the fluorescence measurement for colonies of the coral *S. pistillata* growing in shallow (2 m) and deeper (11 m) waters of the Red Sea [5]. The shallow-growing corals showed significantly lower maximum quantum yields ( $\Delta F/Fm'$  measured during nighttime, equivalent to Fv/Fm) than the deeper growing

ones. It was aslo occurred in other coral species of *F. favus* which grew at 5 m and 20 m water depth of the Red Sea [10]. The spatial pattern of photochemical efficiency of zooxanthellae was related to the light conditions at different water depths. The values of photosynthetic active radiation of the shallow corals were higher than that in the deep waters.

Symbiotic zooxanthellae of corals grew up in the same depth had obvious interspecific differences in photochemical efficiency. Hennige et al. found that there was clear interspecific differences among four dominant massive coral species (*Diploastrea heliopora*, *Favia speciosa*, *F. matthaii* and *Porites lutea*) within the Wakatobi Marine National Park of southeast Sulawesi, Indonesia [12]. Such interspecific differences may be associated with the stability of microhabitat. Piniak and Brown showed that there was no clear inter-specific difference in photosynthetic efficiency in the habitat with a more variable temperature regime, while differences between species were observed only in the more environmentally stable habitat [11].

### 2. Photoacclimation of zooxanthellae and its internal mechanism

Photoacclimation of the zooxanthellae was to acclimate to the changes in irradiance at temporal and spatial scale. Key parameters could describe photoacclimation, for example, the intensity of light-saturated photosynthesis (*Ek*) and the electron transport rate (ETR). Higher values of saturating growth irradiance and the maximum rate of photochemistry were consistent with high light acclimation, and lower values of them corresponding to the slow light acclimation. Symbionts could change the size and location of zooxanthellae, zooxanthellae density and chlorophyll content to adjust the light capturing abilities of symbionts to deal with the spatial and temporal changes in irradiance.

#### 2.1. Photoacclimation phenomena of zooxanthellae

Spatial photoacclimation of zooxanthellae could acclimate to the change of light at different depths. Mass et al. found that the minimum saturating irradiance (Ek) decreased with depth, and in contrast the efficiency of photosynthesis ( $\alpha$ ) increased with depth from the measurement of *S. pistillata* in the Red Sea [13]. Hennige et al. detected that massive P. lutea exhibited a pattern of photoacclimation in which both Ek and rETRmax decreased with increase of optical depth [12]. Suggett et al. confirmed that spatial photoacclimation of corals occurred in many species and demonstrated two different strategies between endemic species and relative dominance of species [14]. Temporal photoacclimation of corals could acclimate to the change of light over time both on a diel and a seasonal scale. For shallow-growing corals in particular, temporal photoacclimation may be of importance in order to not only ensure the effective capture of relatively low irradiances with lower Ek and rETR during the winter but also minimize the effects of high light with higher *Ek* and rETR during the summer [11,15]. Dynamic photoinhibition had been reported for some corals at excess irradiance. Study by Winters et al. [5] showed the values of  $\Delta F/Fm'$  and rETR for the corals growing in shallow water were always lower in the afternoon than during morning hours when measured at similar irradiances. Such photoinhibition was not apparent during the course of the day in the corals growing in deeper water.

#### 2.2. Photoacclimation mechanism of zooxanthellae

Symbiotic zooxanthellae had some mechanisms to acclimate to changing irradiances. They included changes in zooxanthellae density and algal chlorophyll content, and there were differences

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