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Differences in the photoacclimation and photoprotection exhibited by two species of the ciguatera causing dinoflagellate genus, *Gambierdiscus*

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ABSTRACT

In culture, *Gambierdiscus* spp. have been shown to prefer irradiances that are relatively low (≤250 μmol photons m^{-2} s^{-1}) versus those to which they are frequently exposed to in their natural environment (>500 μmol photons m^{-2} s^{-1}). Although several behavioral strategies for coping with such irradiances have been suggested, it is unclear as to how these dinoflagellates do so on a physiological level. More specifically, how do long term exposures (30 days) affect cell size and cellular chlorophyll content, and what is the photosynthetic response to short term, high irradiance exposures (up to 1464 μmol photons m^{-2} s^{-1})? The results of this study reveal that cell size and chlorophyll content exhibited by *G. carolinianus* increased with acclimation to increasing photon flux density. Additionally, both *G. carolinianus* and *G. silvae* exhibited reduced photosynthetic efficiency when acclimated to increased photon flux density. Photosynthetic yield exhibited by *G. silvae* was greater than that for *G. carolinianus* across all acclimation irradiances. Although such differences were evident, both *G. carolinianus* and *G. silvae* appear to have adequate biochemical mechanisms to withstand exposure to irradiances exceeding 250 μmol photons m^{-2} s^{-1} for at least short periods of time following acclimation to irradiances of up to 150 μmol photons m^{-2} s^{-1}.

1. Introduction

Globally, ciguatera fish poisoning (CFP) is the most commonly reported form of phycotoxin-borne illness from seafood consumption (Parsons et al., 2012). Dinoflagellates belonging to the genus *Gambierdiscus* Adachi and Fukuyo are of particular interest because they produce the precursors of ciguatoxins, the toxins responsible for causing CFP outbreaks. Due to the lipophilic nature of ciguatoxins, they bio-accumulate in marine food webs and reach high concentrations in fish (Lewis and Holmes, 1993; Baden et al., 1995; Kibler et al., 2012). People then contract CFP upon consumption of these toxic fish.

Populations of *Gambierdiscus* are often found in shallow (<5 m) tropical waters typically attached to hard substrates and benthic macroalgae (Tindall and Morton, 1998), as well as to the surface-drifting seaweed, *Sargassum* (Bomber et al., 1988a). The photon flux densities found in such environments are highly variable. For example, cloud cover or sediment suspension can cause short term changes in photon flux densities while seasonal and latitudinal changes influence long term averages. Additionally, these environments are often subject to surface irradiances exceeding 2000 μmol photons m^{-2} s^{-1} (Villareal and Morton, 2002). Despite the common occurrence of *Gambierdiscus* cells in environments exposed to high photosynthetically active radiation (PAR) and UV intensities, multiple studies have shown that these organisms have an intolerance to high irradiances (Bomber et al., 1988b; Morton et al., 1992), and achieve maximum growth rates when exposed to relatively low light intensities (Guillard and Keller, 1984; Ballantine et al., 1993; Kibler et al., 2012). Furthermore, studies have shown that photochemistry (Villareal and Morton, 2002) and growth (Kibler et al., 2012) can be inhibited by irradiances far below those recorded in environments where *Gambierdiscus* occurs. These findings have led researchers to suggest that *Gambierdiscus* spp. potentially have multiple mechanisms to protect themselves from high light intensities, including the formation of cell aggregates, the production of light-shielding mucus, and the utilization of three-dimensional structure (i.e., macroalgal thalli) for shade (Indelicato and Watson, 1986; Villareal and Morton, 2002).

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Prior to 1995, there was only one described species of *Gambierdiscus* (*G. toxicus*; Adachi and Fukuyo, 1979). The genus was revised in 2009 (Litaker et al., 2009), and fifteen species are now described (Rhodes et al., 2017 and references therein). Many of the physiological studies conducted prior to revision were conducted on isolates ascribed to *G. toxicus*, but likely involved a species undescribed at the time (confounded by the cryptic nature of *Gambierdiscus* taxonomy; Richlen et al., 2008). As a result, accurate data on species-specific physiology of *Gambierdiscus* are lacking, and such studies need to be repeated using the new species designations based on the recent revisions (Parsons et al., 2012). Because toxicity is known to vary (>100 fold) across species (Babinchak et al., 1986), understanding interspecies eco-physiological variation is critical to understanding the dynamics of CFP outbreaks.

One such study conducted utilizing cultures identified with the revised taxonomic criteria was by Kibler et al. (2012) who examined seven species of *Gambierdiscus* (and one species of *Fukuyoa*) grown in a broad range of light intensities. Their results revealed that while all seven species achieved maximum growth rates at relatively low irradiances, three of the species did not survive irradiances in excess of 250 μmol photons $m^{-2} s^{-1}$. Conversely, the remaining four species maintained growth at irradiances up to 650 μmol photons $m^{-2} s^{-1}$. As this study did not provide three-dimensional structure as a protective measure from high irradiances (>250 μmol photons $m^{-2} s^{-1}$), the results suggest that some of these species may adapt to and withstand high irradiances for extended periods of time on the biochemical level.

Villareal and Morton (2002) utilized cell-specific pulse amplitude modulated (PAM) fluorometry to study the influence of shading on the photosynthetic efficiency of *Gambierdiscus toxicus* (pre-revision designation). They found that diurnal changes in photosynthetic yield were more attenuated in field (shaded) samples versus incubated samples, and that the incubated samples exhibited a more pronounced decrease in yield at mid-day. Additionally, photosynthetic yields were lower in cultures exposed to high (383 μmol photons $m^{-2} s^{-1}$) versus low irradiances (73 μmol photons $m^{-2} s^{-1}$). They concluded that *Gambierdiscus* cells benefit from shading provided by host macroalgae.

Photosynthetic systems and their components are tightly coupled. Therefore, any changes made to one part of a system will affect the other components (Dietzel et al., 2008). Because exposure to abiotic factors such as sunlight are highly variable in nature, both in the long and short-term, all photosynthetic organisms have evolved regulatory responses to cope with exposure to continually variable light intensities. Among these responses is non-photochemical quenching (NPQ) in which the light harvesting complex (LHC) is protected from exposure to excess light energy on short time scales. Non-photochemical quenching consists of several components which are initiated hierarchically in relation to the time it takes to excite and relax each process. In the short-term, energy dependent quenching (qE) is the primary photoprotective process expressed by both plants and algae (Dietzel et al., 2008). This process involves the dissipation of energy as heat through initiation of the xanthophyll cycle and takes seconds to relax (Müller et al., 2001). The secondary NPQ mechanism, state-transition quenching (qT), involves the redistribution of energy between photosystems II and I. This is executed by the manipulation and lateral movement of part of the photosystem II light harvesting complex between photosystems. This process takes minutes to relax and is therefore less plastic and utilized secondarily to qE (Müller et al., 2001; Dietzel et al., 2008). As the methods utilized in the current study do not distinguish between qE and qT, for simplification these processes will be referred to together as NPQ.

Algae and higher plants are also exposed to light conditions that vary over longer time scales that can be brought on by seasonal change as well as vertical and latitudinal migration. Therefore, they have evolved a tertiary long-term response (LTR) to light which involves adjustment of the photosystem stoichiometry (Dietzel et al., 2008). Long-term response differs from NPQ in that it is not purely post-translational, but rather involves changes in photosystem gene expression and the accumulation of Chl *a* and Chl *b* (where applicable). Although LTR occurs over long time periods, the adaptations affect other associated cellular processes like NPQ, and therefore the long-term light history of algae can influence their fitness regarding short-term fluctuations in irradiance (Dietzel et al., 2008). Long-term response can easily be studied in laboratory conditions and is synonymous to light acclimation (Aro and Andersson, 2001; Dietzel et al., 2008).

The data generated by Villareal and Morton (2002) provide preliminary evidence of the effects that LTR has on other photoprotective processes. While their experimental design was appropriate for testing their hypothesis (i.e., *Gambierdiscus* benefits from shade), certain manipulations and additions to their method would provide a more quantitative analysis of the role that LTR plays in photosynthetic capacity and photoprotection over shorter time scales. The goal of this study, therefore, was to explore how expression/magnitude of photoprotective mechanisms such as the components of NPQ are influenced by LTR and how they pertain to the ecology of CFP.

2. Methods

Cultures of *Gambierdiscus carolinianus* (EFM1) and *G. silvae* (Tenn23) were isolated from coastal waters of Long Key, Florida (24°46′17.92“N, 80°45′33.85“W). Both cultures were identified genotypically by Mindy Richlen (Woods Hole Oceanographic Institution) using methods outlined in Xu et al. (2014). Cultures were grown and maintained in 50 mL borosilicate culture tubes containing modified K-media (no copper, TRIS (buffer), or silica). Cultures were pre-acclimated for at least six months at 25 °C and irradiances of approximately 70 μmol photons $m^{-2} s^{-1}$ on a 12:12 h light:dark cycle.

Experimental conditions were consistent with pre-experimental conditions aside from modifications to irradiance exposure. Cell counts were conducted on 6 × 30 μL drops of vortexed culture on an Olympus IX71 inverted microscope using transmitted light at a magnification of 40×. The counts from each of the 6 drops were then averaged and multiplied by 33.33 to convert data to cells m^{-1}L. Additionally, culture fluorescence was measured *in situ* using a Turner 10-AU fluorometer. Following vortexing, cells were observed under the microscope and no cellular damage was evident. This assessment was confirmed by the observation of swimming and pulsing of the transverse flagellum. Likewise, cells continued to maintain stable exponential growth following vortexing. Although the counting method was unorthodox, it was deemed necessary due to constraints regarding cell culture concentrations and volumes. Because *Gambierdiscus* cells are large and benthic in nature, they clump on the surface of the culture vessel rather than occupying the entire volume of culture. This leads to clustering and self-shading at relatively low cell concentrations. Therefore, it was necessary to keep culture populations at a minimum (~150–1000 cells m^{-1}L). The resultant growth rates are assumed to be accurate, as they were similar to those reported in previous studies (Kibler et al., 2012).

2.1. Light driven growth experiments

The light driven growth experiments involved the incubation of 3 × replicate cultures in each of 6 treatments. Treatments consisted