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

Alexander K. Leynse[1,*]

Michael L. Parsons[1]

Serge E. Thomas[1]

1 - Coastal Watershed Institute, Florida Gulf Coast University, 10501 FGCU Blvd South, Fort Myers, FL 33965

* Corresponding author at: University of South Alabama, Dauphin Island Sea Lab, 101 Bienville Boulevard, Dauphin Island Alabama, USA 36528

Highlights:

1. Ciguatera fish poisoning (CFP) is a common form of seafood poisoning.
2. Toxins that cause CFP are produced by the dinoflagellate, *Gambierdiscus*.
3. Light plays an enormous role in the ecology of *Gambierdiscus* and CFP.
4. The strategies of coping with various photon flux densities varies across species of *Gambierdiscus.*
5. The strategies of coping with various photon flux densities exhibited by *Gambierdiscus* spp. are novel.
6. The benthic and epiphytic nature of *Gambierdiscus* can be in part attributed to these dinoflagellates preference for low light intensities.

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}.

Keywords: ciguatera, photosynthesis, harmful algal blooms, HABs

Highlights:

1. Ciguatera fish poisoning (CFP) is a common form of seafood poisoning.
2. Toxins that cause CFP are produced by the dinoflagellate, *Gambierdiscus*.
3. Light plays an important role in the ecology of *Gambierdiscus* and CFP.
4. The strategies of coping with various photon flux densities varies across species of *Gambierdiscus.*
5. The strategies of coping with various photon flux densities exhibited by *Gambierdiscus* spp. are novel.
6. The benthic and epiphytic nature of *Gambierdiscus* can be in part attributed to these dinoflagellates preference for low light intensities.

54

55

56 1. INTRODUCTION

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

64

65 *Gambierdiscus* spp. are often found in shallow (< 5m) tropical waters typically attached to hard
66 substrates and benthic macroalgae (Tindall and Morton, 1998), as well as to the surface-drifting
67 seaweed, *Sargassum* (Bomber *et al*., 1988a). The photon flux densities found in such
68 environments are highly variable. For example, cloud cover or sediment suspension can cause
69 short term changes in photon flux densities while seasonal and latitudinal changes influence long
70 term averages. Additionally, these environments are often subject to surface irradiances
71 exceeding 2,000 µmol photons$\cdot m^{-2} \cdot s^{-1}$ (Villareal and Morton, 2002). Despite the common
72 occurrence of *Gambierdiscus* cells in environments exposed to high photosynthetically active
73 radiation (PAR) and UV intensities, multiple studies have shown that these organisms have an
74 intolerance to high irradiances (Bomber *et al*., 1988b; Morton *et al*., 1992), and achieve
75 maximum growth rates when exposed to relatively low light intensities (Guillard and Keller,
76 1984; Ballantine *et al.*, 1993; Kibler *et al.* 2012). Furthermore, studies have shown that

77 photochemistry (Villareal and Morton, 2002) and growth (Kibler *et al*., 2012) can be inhibited by
78 irradiances far below those recorded in environments where *Gambierdiscus* occurs. These
79 findings have led researchers to suggest that *Gambierdiscus* spp. potentially have multiple
80 mechanisms to protect themselves from high light intensities, including the formation of cell
81 aggregates, the production of light-shielding mucus, and the utilization of three-dimensional
82 structure (i.e., macroalgal thalli) for shade (Indelicato and Watson, 1986; Villareal and Morton,
83 2002).
84
85 Prior to 1995, there was only one described species of *Gambierdiscus* (*G. toxicus*; Adachi and
86 Fukuyo, 1979). The genus was revised in 2009 (Litaker *et al*. 2009), and fifteen species are now
87 described (Rhodes *et al*. 2017 and references therein). Many of the physiological studies
88 conducted prior to revision were conducted on isolates ascribed to *G. toxicus*, but likely involved
89 a species undescribed at the time (confounded by the cryptic nature of *Gambierdiscus* taxonomy;
90 Richlen *et al*. 2008). As a result, accurate data on species-specific physiology of *Gambierdiscus*
91 are lacking, and such studies need to be repeated using the new species designations based on the
92 recent revisions (Parsons *et al.*, 2012). Because toxicity is known to vary (>100 fold) across
93 species (Babinchak *et al*., 1986), understanding interspecies eco-physiological variation is
94 critical to understanding the dynamics of CFP outbreaks.
95
96 One such study conducted utilizing cultures identified with the revised taxonomic criteria was by
97 Kibler *et al*. (2012) who examined seven species of *Gambierdiscus* (and one species of *Fukuyoa*)
98 grown in a broad range of light intensities. Their results revealed that while all seven species
99 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).

146 The data generated by Villareal and Morton (2002) provide preliminary evidence of the effects
147 that LTR has on other photoprotective processes. While their experimental design was
148 appropriate for testing their hypothesis (i.e., *Gambierdiscus* benefits from shade), certain
149 manipulations and additions to their method would provide a more quantitative analysis of the
150 role that LTR plays in photosynthetic capacity and photoprotection over shorter time scales. The
151 goal of this study, therefore, was to explore how expression/magnitude of photoprotective
152 mechanisms such as the components of NPQ are influenced by LTR and how they pertain to the
153 ecology of CFP.
154
155 2. METHODS
156 Both the *Gambierdiscus carolinianus* (EFM1) and *G. silvae* (Tenn23) cultures used in this study
157 were isolated from coastal waters of Long Key, Florida (24°46'17.92"N, 80°45'33.85"W). Both
158 cultures were identified genotypically by Mindy Richlen (Woods Hole Oceanographic
159 Institution) using methods outlined in Xu *et al*. (2014). Cultures were grown and maintained in
160 50mL borosilicate culture tubes containing modified K-media (no copper, TRIS (buffer), or
161 silica). Cultures were pre-acclimated for at least six months at 25°C and irradiances of
162 approximately 70 µmol photons·m^{-2}·s^{-1} on a 12:12 hour light:dark cycle.
163
164 Experimental conditions were consistent with pre-experimental conditions aside from
165 modifications to irradiance exposure. Cell counts were conducted on 6 × 30 µL drops of
166 vortexed culture on an Olympus IX71 inverted microscope using transmitted light at a
167 magnification of 40×. The counts from each of the 6 drops were then averaged and multiplied by
168 33.33 to convert data to cells·mL^{-1}. Additionally, culture fluorescence was measured *in situ* using

169 a Turner 10-AU fluorometer. Following vortexing, cells were observed under the microscope
170 and no cellular damage was evident. This assessment was confirmed by the observation of
171 swimming and pulsing of the transverse flagellum. Likewise, cells continued to maintain stable
172 exponential growth following vortexing. Although the counting method was unorthodox, it was
173 deemed necessary due to constraints regarding cell culture concentrations and volumes. Because
174 *Gambierdiscus* cells are large and benthic in nature, they clump on the surface of the culture
175 vessel rather than occupying the entire volume of culture. This leads to clustering and self-
176 shading at relatively low cell concentrations. Therefore, it was necessary to keep culture
177 populations at a minimum (~150-1,000 cells/mL). The resultant growth rates are assumed to be
178 accurate, as they were similar to those reported in previous studies (Kibler *et al*., 2012).
179
180 2.1 Light driven growth experiments
181 The light driven growth experiments involved the incubation of 3 × replicate cultures in each of
182 6 treatments. Treatments consisted of incubation at irradiances of approximately 15, 30, 60, 80,
183 100, and 150 µmol photons·m^{-2}·s^{-1} for *G. carolinianus* and 30, 50, 70, 100, and 150 µmol
184 photons·m^{-2}·s^{-1} for *G. silvae*. Cultures were acclimated to conditions for at least 3 generations
185 over approximately three to four weeks (depending on the growth rate sustained by the
186 acclimation irradiances). Cell counts and relative fluorescence units (RF) were determined using
187 the methods outlined above. Following acclimation, growth rates were calculated over a 10-day
188 period to reduce error caused by the relatively slow growth rate of both *Gambierdiscus* species.
189 Fit curves were applied to the light driven growth data using the model by Eilers and Peeters
190 (1988).
191

2.2 Long term photoacclimation experiments

For each species, triplicate cultures were grown and acclimated to one of three acclimation irradiances (AI) determined from the results of the light driven growth experiments described above. Acclimation irradiances consisted of a low irradiance (30 µmol photons·m^{-2}·s^{-1}) where growth was inhibited as a result of light limitation, an optimal irradiance (100 µmol photons·m^{-2}·s^{-1}) where maximum light driven growth was first achieved along the curve, and a high irradiance (150 µmol photons·m^{-2}·s^{-1}) where growth was not inhibited, but also did not exceed that of the growth achieved at optimal irradiance. Although the high AI did not result in a reduced growth rate, it was anticipated that suboptimal cellular LTR arrangements were occurring due to the excess irradiance.

Cultures were acclimated to the AIs for 30 days. During this period, cell counts and culture fluorescence were monitored in the same manner as described previously. Although an additional AI of higher irradiance was desirable (i.e., at an intensity that would have slightly reduced growth), suitable alternative light sources were cost prohibitive. Cell volumes were also measured during these experiments, using an ocular micrometer on the Olympus IX71 microscope. Length, width, and height measurements of 12 randomly selected cells from each culture were taken and recorded for this purpose.

Following the 30-day acclimation period, photosynthesis irradiance (P-E) curves were generated for each species, acclimated to each of the three AIs (18 × total cultures), using a Walz Phyto-PAM phytoplankton analyzer set up in the following manner. The Phyto-PAM measuring head contains an optical port which receives a cylindrical quartz cuvette containing sample (2.5 mL).

Measuring and actinic light emitting diodes (LEDs) were placed in two arrays around the optical port and were focused at the bottom of the sample cuvette. A type H6779-01, Hamamatsu photomultiplier detector with high red sensitivity and a special filter set to remove excess excitation light was seated below the cuvette for fluorescence readings. Due to a combination of time constraints (i.e. P-E curve duration, the 12 hour light cycle, triplicate culture, identical acclimation duration) and equipment constraints (i.e. single Phyto-PAM and limited incubator space) replicate cultures had to be analyzed on the same day but at different times during the light cycle. To avoid this issue interfering with comparisons between AIs and species, each replicate was exposed to the P-E curve at a different time block during the light cycle and averaged (i.e. replicate #1: morning (8-11 am); replicate #2: mid-day (11:30 – 2:30 pm); and replicate #3 afternoon (3-6 pm). Aliquots of culture diluted to contain approximately 400 $\mu g \cdot L^{-1}$ chlorophyll *a*, were placed into the measuring head. Photosynthesis-irradiance curves were then generated for each of the experimental cultures, and consisted of a step-wise exposure to increasing irradiances; 16, 32, 64, 164, 264, 464, 664, 864, 1264, and 1464 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$, followed by a final step at 64 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$, which was used as a recovery measurement. The duration of exposure at each step was 15 min., at the end of which a fluorescence measurement (Fo') was recorded, followed by a maximum fluorescence (Fm') measurement. Maximum fluorescence was recorded by exposing the cells to a saturating pulse of actinic light (2600 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$) for 0.19 seconds. The dark acclimated maximum fluorescence (Fm) was measured at 32 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$. From the P-E curve data, values for photosynthetic yield were calculated:

$$yield = \frac{Fm' - Fo}{Fm'}$$

As well as values for NPQ:

$$NPQ = \frac{Fm - Fm'}{Fm'}$$

Fluorescence data from the P-E curves were averaged for each of the three acclimation irradiances.

2.3 Statistics

IBM SPSS statistics software (V. 22.0) was used for all statistical analyses. One-way ANOVA and Tukey Post Hoc tests were used to compare mean cell size, RF per cell, and yield recovery across AIs and species. Relative fluorescence per cell data were transformed to achieve normality by applying a 4^{th} root function to the data. Photosynthetic yield data generated from P-E curves were normal by the Kolmogorov-Smirnov test. Statistical comparisons between P-E curves were made using linear mixed model analysis.

3. RESULTS

The light driven growth experiments revealed that both *G. carolinianus* and *G. silvae* achieved maximum growth rates at irradiances of approximately 100 µmol photons·m^{-2}·s^{-1}. Growth exhibited by both *G. carolinianus* ($p = 0.907$) and *G. silvae* ($p = 0.317$) did not significantly change with exposure to increasing irradiances up to 150 µmol photons·m^{-2}·s^{-1} (Fig. 1).

Cell volume comparisons revealed that cell sizes for *G. silvae* did not significantly differ across AIs. Cell volumes calculated for *G. carolinianus* from the low AI treatments, however, were

258 significantly smaller than those acclimated to optimal ($p = 0.038$) and high ($p = 0.016$) AIs (Fig.
259 2).

260 Relative fluorescence per cell comparisons show that *G. silvae* did not yield any significant
261 differences in RF across AIs (Fig. 3). Interestingly, while the RF values of *G. carolinianus* were
262 statistically similar to those of *G. silvae* from the low AI treatment, RF per cell values for *G.*
263 *carolinianus* significantly increased with increasing AI intensity (Low<Opt, $p = 0.02$; Low<High,
264 $p = 0.000$; Opt>High, $p = 0.001$).
265
266 Comparisons of the grouped mean photosynthetic yield generated from P-E curves (Fig. 4 & Table
267 1) revealed significant differences between all AIs for both *G. carolinianus* and *G. silvae* (all p-
268 values = 0.00). Additionally, *G. silvae* exhibited a significantly higher yield than *G. carolinianus*
269 for all AIs (high, $p = 0.00$; optimal, $p = 0.00$; low, $p = 0.032$). Likewise, these results are easily
270 distinguished by graphically comparing the P-E curve data (Fig. 5). While no significant
271 differences were found for the recovery of photosynthetic yield following exposure to the P-E
272 curve, graphically it appears that recovery decreased with increasing AI for both species (Fig. 6).
273 Estimates of NPQ were calculated from the P-E curve data for both *G. carolinianus* and *G. silvae*
274 (Fig. 7). While the calculation of NPQ is simple, the equation exaggerates the error associated with
275 the replicate Fm' and Fm data, resulting in negative values (Fig. 7) that should be disregarded as
276 truly negative. Consequently, the calculated NPQ values do not meet assumptions for statistical
277 testing. Distinct differences are graphically apparent, however, across *G. carolinianus* AIs.
278 Additionally, the calculated NPQ exhibited by *G. silvae* from each AI are closely grouped and fall
279 below the lowest NPQ exhibited by *G. carolinianus*.
280

281 4. DISCUSSION

282 The results of this study demonstrated that significant differences in yield across AIs for both
283 observed species exist. This finding suggests that LTR induced by light history (e.g. AI)
284 influences the expression of one or both NPQ components. Interestingly, the lowest
285 photosynthetic yields generated from the P-E curves were 0.11 for *G. carolinianus* and 0.28 for
286 *G. silvae* (recorded at 1264 and 1464 μmol photons $\cdot m^{-2} \cdot s^{-1}$ following 90 and 105 minutes of
287 exposure to P-E curve irradiances greater than 100 μmol photons$\cdot m^{-2} \cdot s^{-1}$, respectively). Both
288 yields are relatively high compared to those reported by Villareal and Morton (2002), where cells
289 acclimated to both 73 and 383 μmol photons$\cdot m^{-2} \cdot s^{-1}$ for just one week possessed yields of ≈ 0
290 when recorded at 660 μmol photons$\cdot m^{-2} \cdot s^{-1}$ following only 5 minutes of exposure to rapid light
291 curve (RLC) irradiances exceeding 100 μmol photons$\cdot m^{-2} \cdot s^{-1}$. While the cause for this difference
292 is unclear, it is possible that factors such as speciation and/or the relatively short duration of the
293 RLC administered by Villareal and Morton (2002) may have been responsible.
294
295 Photosynthetic yield has not only been used to measure the photochemical activity of
296 photosynthetic systems (Kolber *et al*., 1988; Greene *et al*., 1992; Geider *et al*., 1993; Kolber and
297 Falkowski, 1993; Kolber *et al*., 1994), but has also been used as a proxy for determining nutrient
298 limitation. Many studies have reported that depressed signals of photosynthetic yield are a result
299 of nutrient limitation (Kolber *et al*., 1990; Geider *et al*., 1993; Kolber *et al*., 1994; Behrenfeld *et*
300 *al*., 1996). The cultures examined by Villareal and Morton (2002) were isolated from tropical
301 oligotrophic waters and cultured in laboratory conditions for a total of 17 days prior to
302 experimentation, whereas the *G. carolinianus* and *G. silvae* cultures used in this study were
303 maintained in culture for at least six months prior to the initiation of acclimation to AIs.

Therefore, it is possible that the differences between the long-term nutrient history of the cultures used in this study and that conducted by Villareal and Morton (2002) could explain the inconsistencies in yield between studies.

It is well known that phytoplankton will increase cellular chlorophyll content as irradiance decreases (MacIntyre *et al*., 2002; Dubinsky and Stambler, 2009). Typically, the increase in cellular chlorophyll at low irradiances is thought to provide improved light harvesting while the decrease in cellular chlorophyll at high irradiances is attributed to dilution via increased cell division rates (Post *et al*., 1984; Prézelin *et al*., 1991). The opposite was exhibited by *G. carolinianus*. When acclimated to light-limiting conditions, *G. carolinianus* exhibited a decrease in cell size (Fig. 2) and cellular chlorophyll content (Fig.3). Conversely, when acclimated to higher than optimal irradiances, *G. carolinianus* exhibited increased cellular chlorophyll concentrations. Although this response to light is unusual, it may have its benefits. For example, too much cellular chlorophyll can result in the shading of some of the chlorophyll molecules thus resulting in a less efficient light harvesting process (Berner *et al*., 1989). Larger cells are especially vulnerable to this phenomenon due to the reduced surface area to volume ratio (Kirk, 1986; Geider *et al*., 1986). Therefore, it is possible that in light limiting regimes such as those created in this study, *G. carolinianus* may sustain a more efficient cellular carbon (C) allocation by reducing cellular chlorophyll concentrations. Ultimately, this response would allow *G. carolinianus* to sustain steady growth in a light limited environment. Because *G. carolinianus* exhibits a relatively slow maximum growth rate, it is possible that the cellular chlorophyll is not diluted by cell division, resulting in an increased cellular chlorophyll content when acclimated to higher than optimal irradiances. Another possibility is that the increase in cellular chlorophyll

concentration increases the robustness of photon capture at high photon flux densities, to compensate for the reduced photosynthetic efficiency accompanied by acclimation to high irradiances.

Growth rate is a parameter used to assess fitness in microalgal cultures. Light driven growth curves allow investigators to compare the fitness of cultures across a range of light intensities. Multiple physiological adaptations influence light dependent growth (i.e., chlorophyll content, photosynthetic yield, cellular metabolism, C partitioning, and abiotic variables). Both species observed in this study exhibited similar light driven growth curves (Fig. 1). Interestingly, these species responded to changing AIs differently regarding cellular RF, cell volume, and photosynthetic yield. Across AIs, significant changes in cell size (Fig. 2) and RF per cell (Fig. 3) were observed for *G. carolinianus*, but not *G. silvae*, leading to some interesting interpretations.

While both species exhibited a serial decline in photosynthetic yield with increasing AI intensity, the photosynthetic yield exhibited by *G. silvae* across AIs was significantly greater than that of *G. carolinianus* for each corresponding AI (Fig.4). Additionally, the NPQ values calculated from P-E curves provide a visual representation of the marked differences in NPQ expressed by the two species (Fig. 7). The cell size exhibited by *G. silvae* in the low AI was consistent across all AIs, making this species, on average, smaller than *G. carolinianus*. Meanwhile, the cellular RF expressed by *G. silvae* did not significantly vary across AIs suggesting a consistent cellular chlorophyll concentration (Fig.3).

The yield recovery measurements (Fig. 6) depict an apparent trend in reduced recovery of photosynthetic yield across increasing AI intensities for both species. This trend is possibly a result of induced photoinhibition (qI), which results from photodamage, and unlike NPQ components, can take hours to recover. Photoinhibition can be very costly to photosynthetic organisms and is expected to occur following prolonged exposure to irradiances such as those experienced later in the P-E curve trials. In comparison, *G. silvae* appeared to recover yield more so than *G. carolinianus* following exposure to P-E curves (Fig. 6). This result is somewhat puzzling, considering the greater apparent expression of NPQ by *G. carolinianus*.

Examination of the changes each species exhibited in succession from low to high AI reveals an interesting scenario. In the low AI treatment, growth rate, yield, cell size, and cellular RF were all statistically indistinguishable between *G. carolinianus* and *G. silvae*. As AI increased to optimal irradiance, a higher growth rate was observed for each species. Additionally, LTR resulted in a reduced photosynthetic yield, more so for *G. carolinianus* than for *G. silvae*. This decrease is likely related, in part, to an increase in NPQ expression, one that appears to be greater for *G. carolinianus* (Fig. 7). Despite the lower yield exhibited by *G. carolinianus*, both species continued to grow at a similar rate (Fig. 1). While both cell size and chlorophyll content of *G. silvae* remained constant, both parameters increased for *G. carolinianus*. As cultures progressed to the high AI treatment, physiological changes continued to change in a similar manner. In the transition from optimal to high AI conditions, growth rate did not increase, but rather remained constant. From this scenario, it appears that *G. silvae* maintains more efficient balance between NPQ and photochemistry overall than *G. carolinianus*. On the other hand, *G. carolinianus* may in part, overcome this potential disadvantage by increasing cellular chlorophyll content to

maintain a higher growth rate and remain competitive. There appears to be a disadvantage to the strategy of *G. carolinianus*, however, as the lower photosynthetic yield expressed during P-E curves suggests that this species is less efficient when assimilating short durations of high intensity light. Additionally, *G. carolinianus* appears to be more prone to qI (Fig. 6). The qI data were not significantly different between species, however, and further investigation will be necessary before any conclusions can be made.

The use of multiple AIs provides insight on how *Gambierdiscus* spp. cope with varying intensities of irradiance over long time scales in nature. For example, *Gambierdiscus* populations accustomed to shallow tropical waters would be consistently exposed to relatively high, potentially damaging irradiances. In such environments, behavioral responses such as utilizing shade from structure, forming cell aggregates, and mucus production (Indelicato and Watson, 1986; Villareal and Morton, 2002), are likely necessary. Likewise, appropriate configuration of photosystem components via LTR to such irradiances would allow cells to optimize carbon assimilation across a range of irradiances. There seems to be a trade-off, however, between irradiances adequate for sustaining maximum growth and lower irradiances that induce less stress to the LHC. For example, populations residing in low light conditions (i.e. deep in the water column or seasonally induced turbid waters) will sustain a higher yield during short term exposure to excessive photon flux densities (> 250 μmol photons$\cdot m^{-2} \cdot s^{-1}$). Therefore, the optimal photon flux densities for *Gambierdiscus* spp. are likely the lowest intensities at which maximal growth is sustained. Such irradiances eliminate unnecessary light induced stress to the LHC, enable cells to maintain maximum growth, and minimally compromise photosynthetic yield during periodic short-term exposure to high irradiances.

While the light management strategies of *G. carolinianus* are somewhat transparent (i.e., increased cellular chlorophyll), the mechanisms by which *G. silvae* sustains a high photosynthetic yield when acclimated to high irradiances is beyond the scope of this study. Further study, may reveal species specific differences in the organization and/or expression of LHC associated proteins (Müller *et al*., 2001).

5. CONCLUSION

In conclusion, this study has revealed that LTR induced by acclimation irradiances influences the photosynthetic response of both *G. carolinianus* and *G. silvae* to shorter duration exposures of varying light intensities. Consequently, cells acclimated to high irradiances (>100 µmol photons·m^{-2}·s^{-1}) will maintain maximum growth in static lighting conditions. While cells acclimated to lower irradiances (<100 µmol photons·m^{-2}·s-1) are more likely to sustain limited growth in static lighting, they are more efficient when exposed to short durations of high photon flux densities.

Although relatively low irradiances (100 µmol photons·m^{-2}·s^{-1}) are optimal for the species observed in this study, the results indicate that both species possess the capability to cope with irradiances far exceeding their optima for longer periods of time than was previously thought (> 60 min.). The cell sizes and chlorophyll content for *G. carolinianus* are plastic and allow for manipulation to achieve improved growth rates at irradiances that are above or below optimal conditions. Although the cell sizes and chlorophyll content of *G. silvae* cells do not appear to be as plastic as those of *G. carolinianus*, this species possesses the ability to maintain a relatively

high photosynthetic yield in irradiances that exceed optimal range, all the while avoiding significant photoinhibition. Understanding how each species copes with varying light intensities over short and long timescales can provide insight to ecological models formulated to predict proliferation of *Gambierdiscus* populations and CFP outbreaks.

Acknowledgements

We would like to thank Adam Catasus and Jeff Zingre for their assistance in the laboratory. We would also like to thank the Blair Foundation Scholarship for their support of student research involved in this project. Funding for this work was provided by NOAA NOS (Cooperative Agreements NA11NOS478-0060 and NA11NOS4780028). This is ECOHAB publication number 846.

References

Aro, E.M., Andersson, B., 2001. Regulation of Photosynthesis. Kluwer Academic Publishers, Dordrecht.

Babinchak, J.A., Jollow, D.J., Voegtline, M.S., Higerd, T.B., 1986. Toxin production by *Gambierdiscus toxicus* isolated from the Florida Keys. Marine Fisheries Review. 48 (4), 53-56.

Baden, D.G., Flemming, L.E., Bean, J.A., 1995. Marine toxins. In: De Wolff, F.A. (Ed.), Handbook of Clinical Neurology: Intoxications of the Nervous System. II. Natural Toxins and Drugs. Elsevier Science, Amsterdam, pp. 141-175.

Ballantine, D.L., Bardales, A.T., Alvey, M.E., 1993. The culture of three dinoflagellate species associated with ciguatera. In: Calumpong H.P., Meñez, E.G., (Eds.), Proceedings of the 2nd RP-USA Phycology Symposium/Workshop, Philippines, National Science Foundation (U.S.)/ Philippine Council for Aquatic and Marine Research & Development, pp. 261-268.

Behrenfeld, M.J., Bale, A.J., Kolber, Z.S., Aiken, J., Falkowski, P.G., 1996. Conformation of iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean. Nature. 383, 508-511.

Berner, T., Wyman, K., Dubinsky, Z., Falkowski, P.G., 1989. Photoadaptation and the 'package' effect in *Dunaliella tertiolecta* (Chlorophyceae). Phycology. 25, 70-78.

Bomber, J.W., Morton, S.L., Babinchak, J.A., Norris, D.R., Morton, J.G., 1988a. Epiphytic dinoflagellates of drift algae – another toxigenic community in the ciguatera food chain. Bulletin of Marine Science. 43, 204-214.

Bomber, J.W., Guillard, R.R.L., Nelson, W.G., 1988b. Roles of temperature, salinity, and light in seasonality, growth, and toxicity of ciguatera-causing *Gambierdiscus toxicus* Adachi *et* Fukuyo (Dinophyceae). Experimental Marine Biology and Ecology. 115, 53-65.

Dietzel, L., Bräutigam, K., Pfannschmidt, T., 2008. Photosynthetic acclimation: State transitions and adjustment of photosystem stoichiometry – functional relationships between short-term and long-term light quality acclimation in plants. FEBS. 275, 1080-1088.

Dubinsky, Z., Stambler, N., 2009. Photoacclimation processes in phytoplankton: mechanisms, consequences, and applications. Aquatic Microbial Ecology. 56, 163-176.

Eilers, P.H.C., Peeters, J.C.H., 1988. A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. Ecological Modeling. 42, 199-215.

Greene, R.M., Geider, R.J., Kolber, Z., Falkowski, P.G., 1992. Iron-induced changes in light harvesting and photochemical energy conversion processes in marine eukaryotic marine algae. Plant Physiology. 100, 565-575.

Geider, R.J., Platt, T., Raven, J.A., 1986. Size dependence of growth and photosynthesis in diatoms: a synthesis. Marine Ecology Progress Series. 30, 93-104.

Geider, R.J., Greene, R.M., Kolber, Z., MacIntyre, H.L., Falkowski, P.G., 1993. Fluorescence assessment of the maximum quantum efficiency of photosynthesis in the western North Atlantic. Deep-Sea Research. 40, 1205-1224.

Guillard, R.R.L., Keller, M.D., 1984. Culturing Dinoflagellates. In. Spector, D.L. (Ed.), Dinoflagellates. Academic Press, Orlando, FL, pp. 391-433.

Indelicato, S.R., Watson, D.A., 1986. Identification of the photosynthetic pigments of the tropical benthic dinoflagellate *Gambierdiscus toxicus*. Marine Fisheries Review. 48, 44-47.

Kibler, S.R., Litaker, R.W., Holland, W.C., Vandersea, M.W., Tester, P.A., 2012. Growth of eight *Gambierdiscus* (Dinophyceae) species: Effects of temperature, salinity, and irradiance. Harmful Algae. 19, 1-14.

Kirk, J.T.O., 1986. Optical properties of picoplankton suspensions. Canadian Bulletin of Fisheries and Aquatic Sciences. 214, 501-520.

Kolber, Z., Zehr, J., Falkowski, P., 1988. Effects of growth, irradiance, and nitrogen limitation on photosynthetic energy conversion in photosystem II. Plant Physiology. 88, 923-929.

Kolber, Z., Wyman, K.D., Falkowski, P.G., 1990. Natural variability in photosynthetic energy conversion efficiency: A field study in the Gulf of Maine. Limnology and Oceanography. 35, 72-79.

Kolber, Z., Falkowski, P.G., 1993. Use of active fluorescence to estimate phytoplankton photosynthesis in situ. Limnology and Oceanography. 38, 1646-1665.

Kolber, Z.S., Barber, R.T., Coale, K.H., Fitzwater, S.E., Greene, R.M., Johnson, L.S., Falkowski, P.G., 1994. Iron limitation of phytoplankton photosynthesis in the equatorial ocean. Nature. 371, 145-149.

Lewis, R.J., Holmes, M.J.,1993. Origin and transfer of toxins involved in ciguatera. Comparative Biochemistry and Physiology. C: Comparative Pharmacology. 106, 615-628.

Litaker, R.W., Vandersea, M.W., Faust, M.A., Kibler, S.R., Chinain, M., Holmes, M.J., Holland, W.C., Tester, P.A., 2009. Taxonomy of *Gambierdiscus* including four new species, *Gambierdiscus caribaeus*, *Gambierdiscus carolinianus*, *Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae). Phycologia, 48, 344-390.

MacIntyre, H.L., Kana, T.M., Anning, T., Geider, R.J., 2002. Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. Phycology. 38, 17-38.

523 Morton, S.L., Norris, D.R., Bomber, J.W., 1992. Effect of temperature, salinity, and light
524 intensity on the growth and seasonality of toxic dinoflagellates associated with ciguatera.
525 Experimental Marine Biology and Ecology. 157, 79-90.
526
527 Müller, P., Li, X.P., Niyogi, K.K., 2001. Non-photochemical quenching. A response to excess
528 light energy. Plant Physiology. 125, 1558-1566.
529
530 Parsons, M.L., Aligizaki, K., Bottein, M.D., Fraga, S., Morton, S., Penna A., Rhodes, L., 2012.
531 *Gambierdiscus* and *Ostreopsis*: Reassessment of the state of knowledge of their taxonomy,
532 geography, ecophysiology, and toxicology. Harmful Algae. 14, 107-129.
533
534 Post, A.F., Dubinsky, Z., Wyman, K., Falkowski, P.G., 1984. Kinetics of light intensity
535 adaptation in a marine plankton diatom. Marine Biology. 83, 231-238.
536
537 Prézelin, B.B., Tilzer, M.M., Schofield, O., Haese, C., 1991. The control of the production
538 process by the physical structure of the aquatic environment with special reference to its optical
539 properties. Aquatic Science. 53, 136-186.
540
541 Rhodes, L., Smith, K.F., Verma, A., Curley, B.G., Harwood, D.T., Murray, S., Kohli, G.S.,
542 Solomona, D., Rongo, T., Munday, R., Murray, S.A., 2017. A new species of *Gambierdiscus*
543 (Dinophyceae) from the south-west Pacific: *Gambierdiscus honu* sp. nov. Harmful Algae, 65,
544 61-70.
545

546 Richlen, M. L., Morton, S. L., Barber, P.H., Lobel, P. S., 2008. Phylogeography, morphological
547 variation and taxonomy of the toxic dinoflagellate *Gambierdiscus toxicus*. Harmful Algae. 7,
548 614-629.
549
550 Tindall, D.R., Morton, S.L., 1998. Community dynamics and physiology of epiphytic/benthic
551 dinoflagellates associated with ciguatera. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.A.
552 (Eds.), Physiological Ecology of Harmful Algal Blooms. NATO ASI Series, Series G:
553 Ecological Sciences 41. Springer-Verlag, Berlin Heidelberg, pp. 293-113.
554
555 Villareal, T.A., Morton, S.L., 2002. Use of cell-specific PAM fluorometry to characterize host
556 shading in the epiphytic dinoflagellate *Gambierdiscus toxicus*. Marine Ecology. 23, 127-14.
557
558 Xu, Y., Richlen, M.L., Morton, S.L., Mak, Y.L., Chan, L.L., Tekiau, A., Anderson, D.M., 2014.
559 Distribution, abundance and diversity of *Gambierdiscus* spp. from a ciguatera-endemic area in
560 Marakei, Republic of Kiribati. Harmful Algae. 34, 56-68.
561
562

563 List of figures

564 Fig. 1. Kinetic light driven growth rates for *G. carolinianus* (closed circles) and *G. silvae* (open
565 circles). Dotted lines represent the applied Eilers and Peeters (1988) model for graphical
566 representation.

567

568 Fig. 2. Cell volumes of *G. carolinianus* (grey) and *G. silvae* (white) following acclimation to
569 high, optimal, and low irradiances. Letters on graph indicate statistically-distinct groups. The
570 asterisk indicates that the average and standard error is only represented by two individual data
571 points.

572

573 Fig. 3. Comparison of the RF per cell exhibited by *G. carolinianus* (grey) and *G. silvae* (white)
574 from each AI. Letters on graph indicate statistically-distinct groups.

575

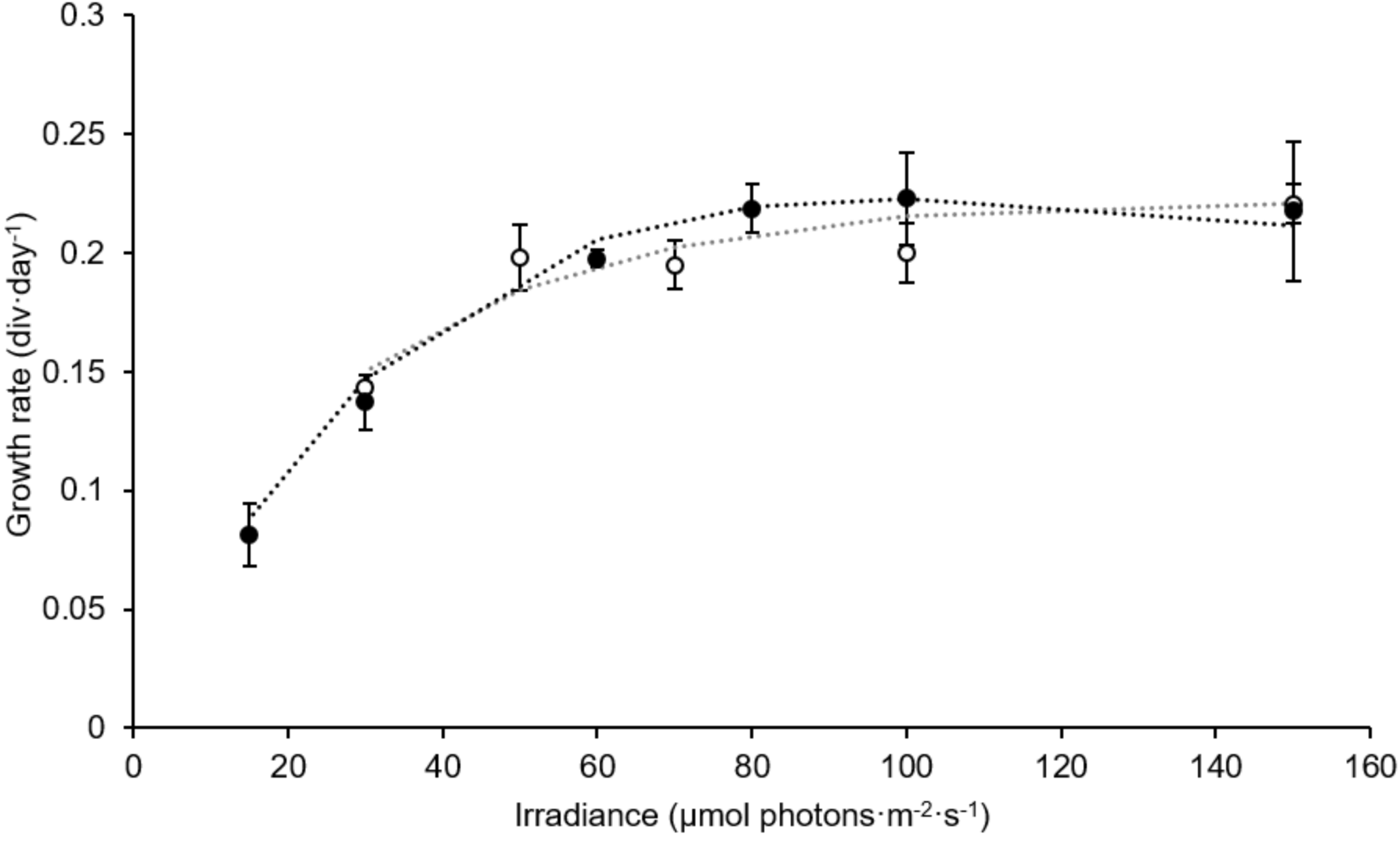
576 Fig. 4. Comparison of the grouped mean photosynthetic yield (e.g. derived from P-E curves) of
577 *G. carolinianus* (grey) and *G. silvae* (white) from each AI. Letters on graph indicate statistically-
578 distinct groups.

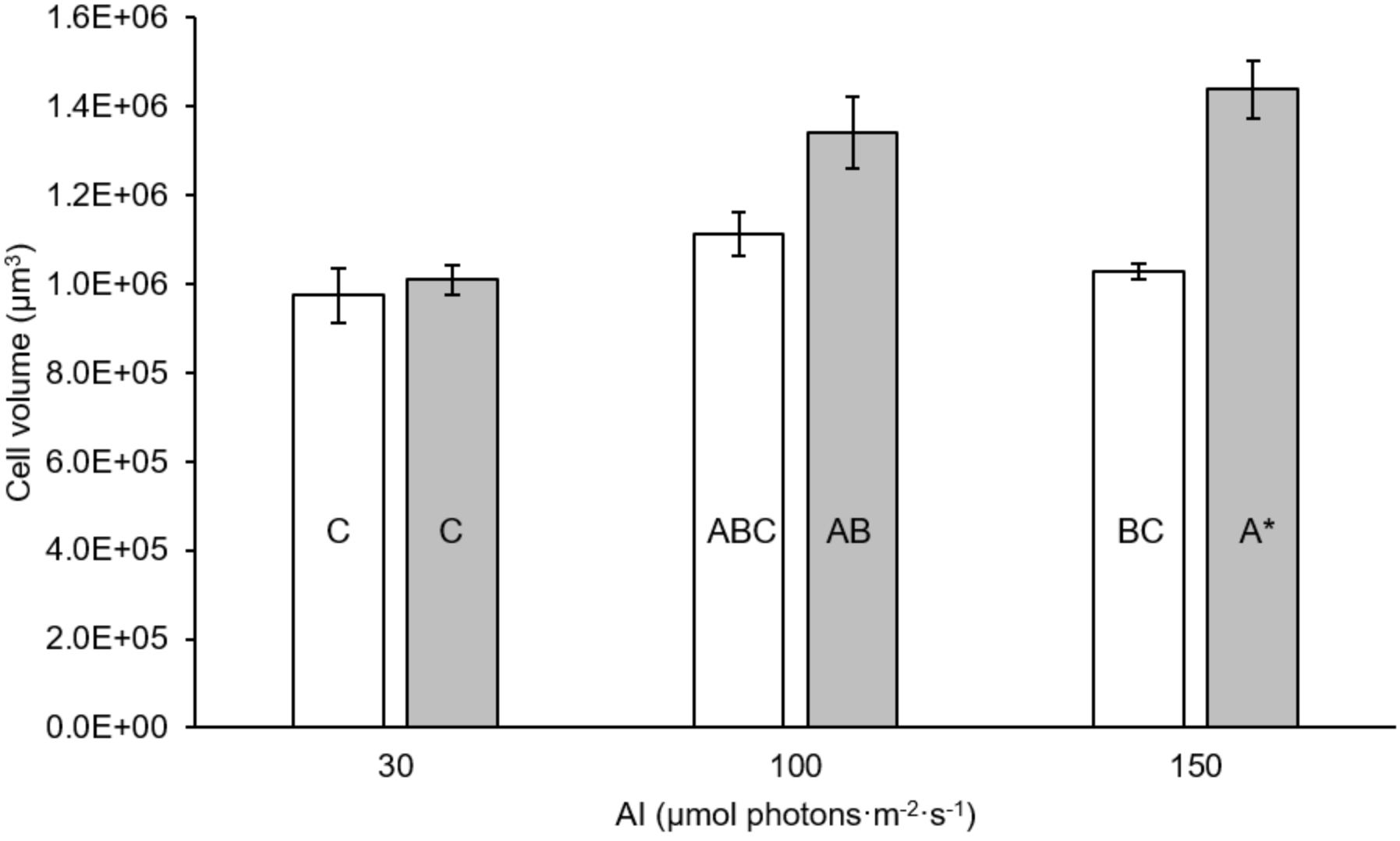
579

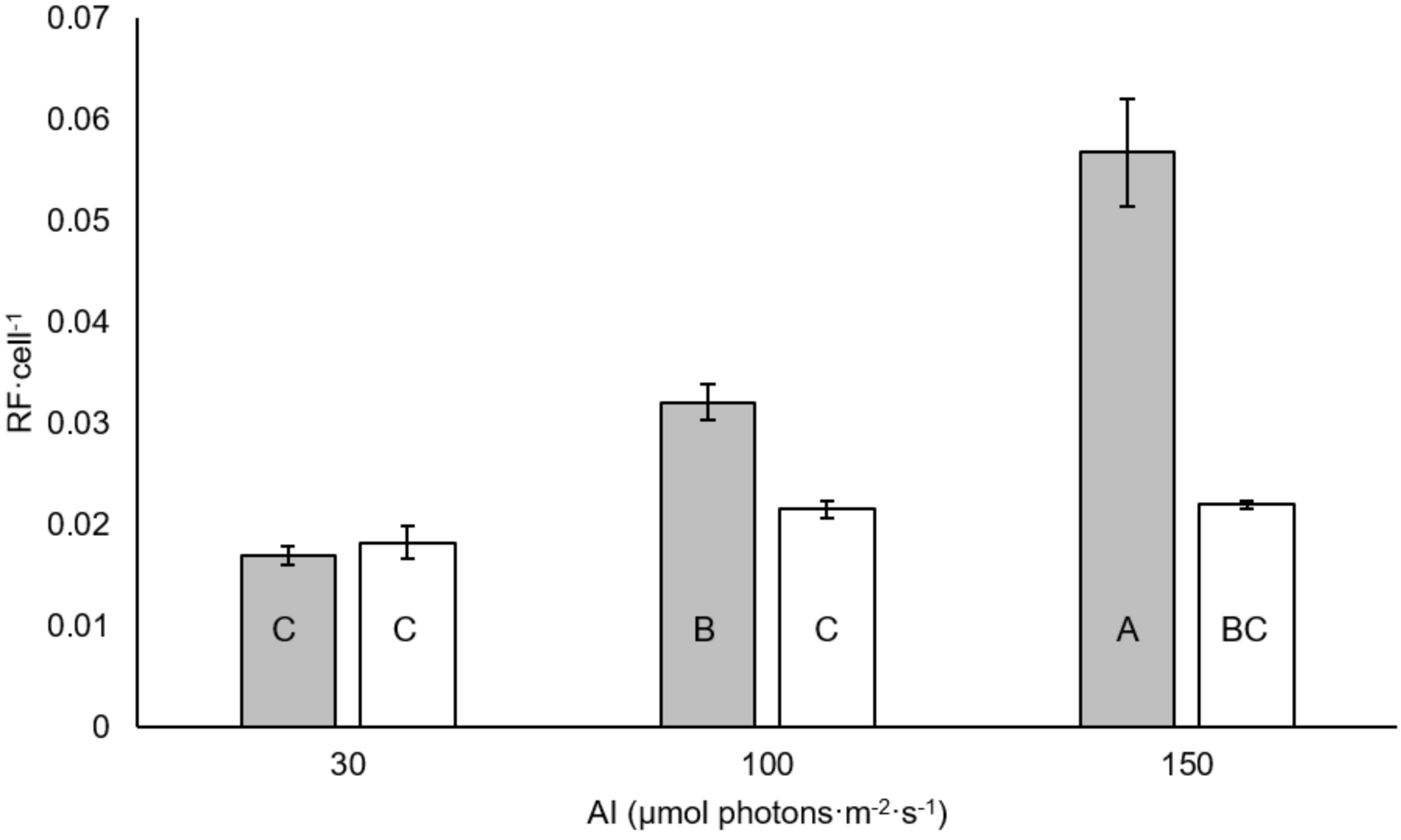
580 Fig. 5. Comparison of the kinetic photosynthetic yield expressed by *G. carolinianus* (closed
581 symbols) and *G. silvae* (open symbols) during P-E curves (15 min. steps and 2.5 hours total
582 exposure time). Corresponding AIs are depicted as follows, low irradiance (triangles), optimal
583 irradiance (circles), and high irradiance (diamonds).

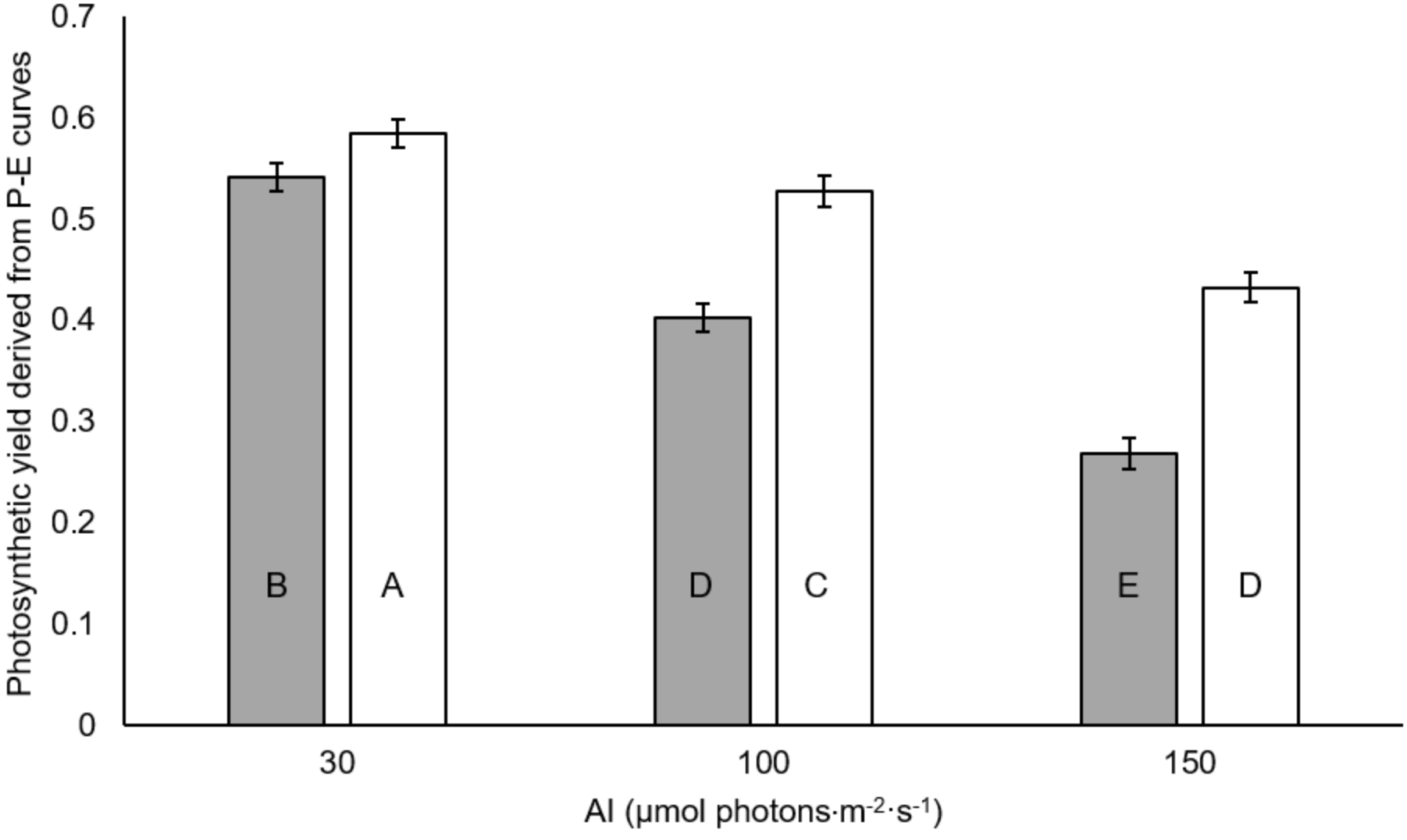
584

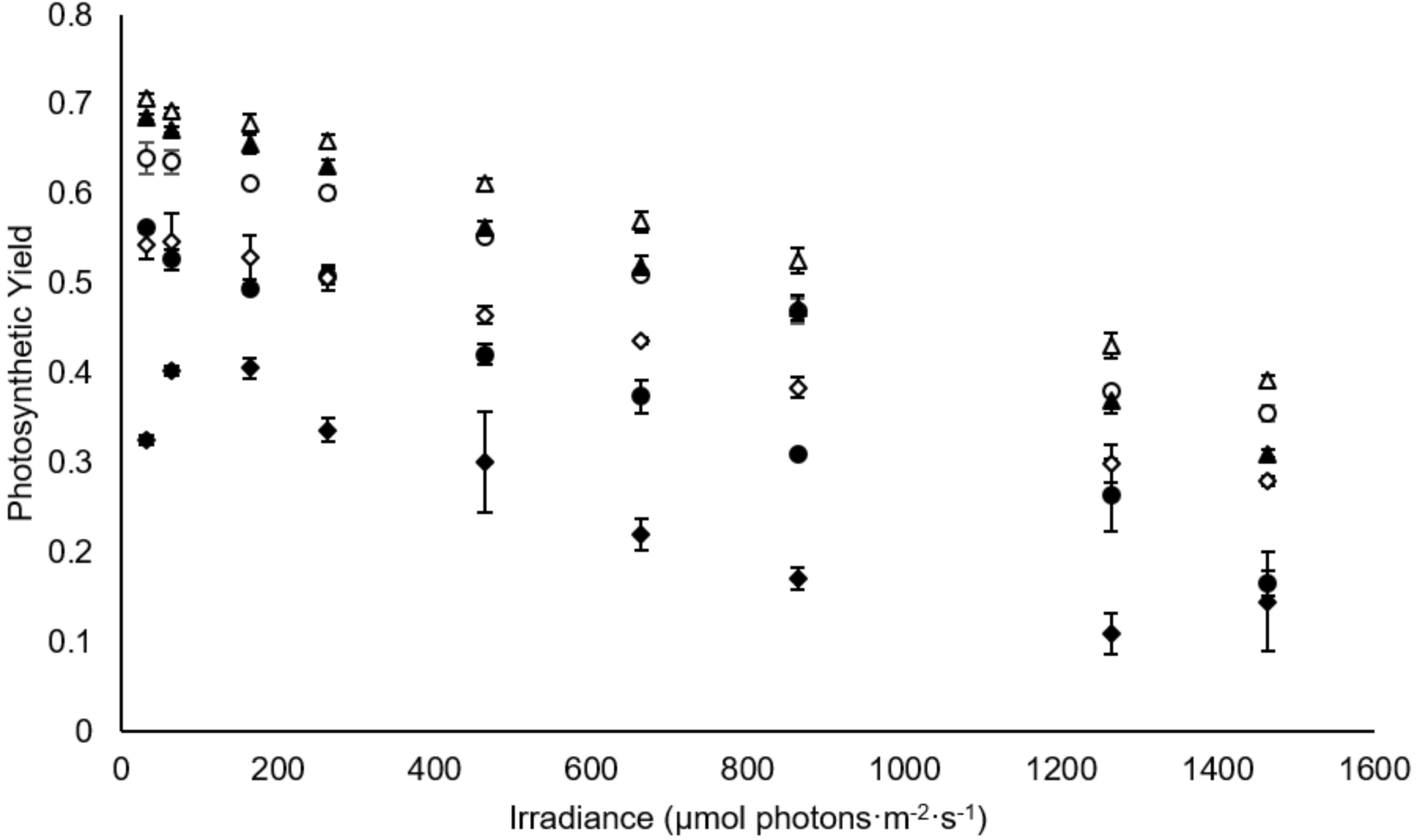
585 Fig. 6. Recovery of photosynthetic yield for *G. carolinianus* (grey) and *G. silvae* (white)
586 following exposure to P-E curve (15 min. steps and 2.5 hours total exposure time). Recovery
587 period consisted of a 15 min. exposure to 64 µmol photons·m^{-2}·s^{-1}. Data were not statistically
588 different.
589
590 Fig. 7. Calculated NPQ exhibited by *G. carolinianus* (AI high: closed diamonds, optimal: closed
591 circles, low: closed triangles) and *G. silvae* (AI high: open diamonds, optimal: open circles, low:
592 open triangles) during P-E curves.

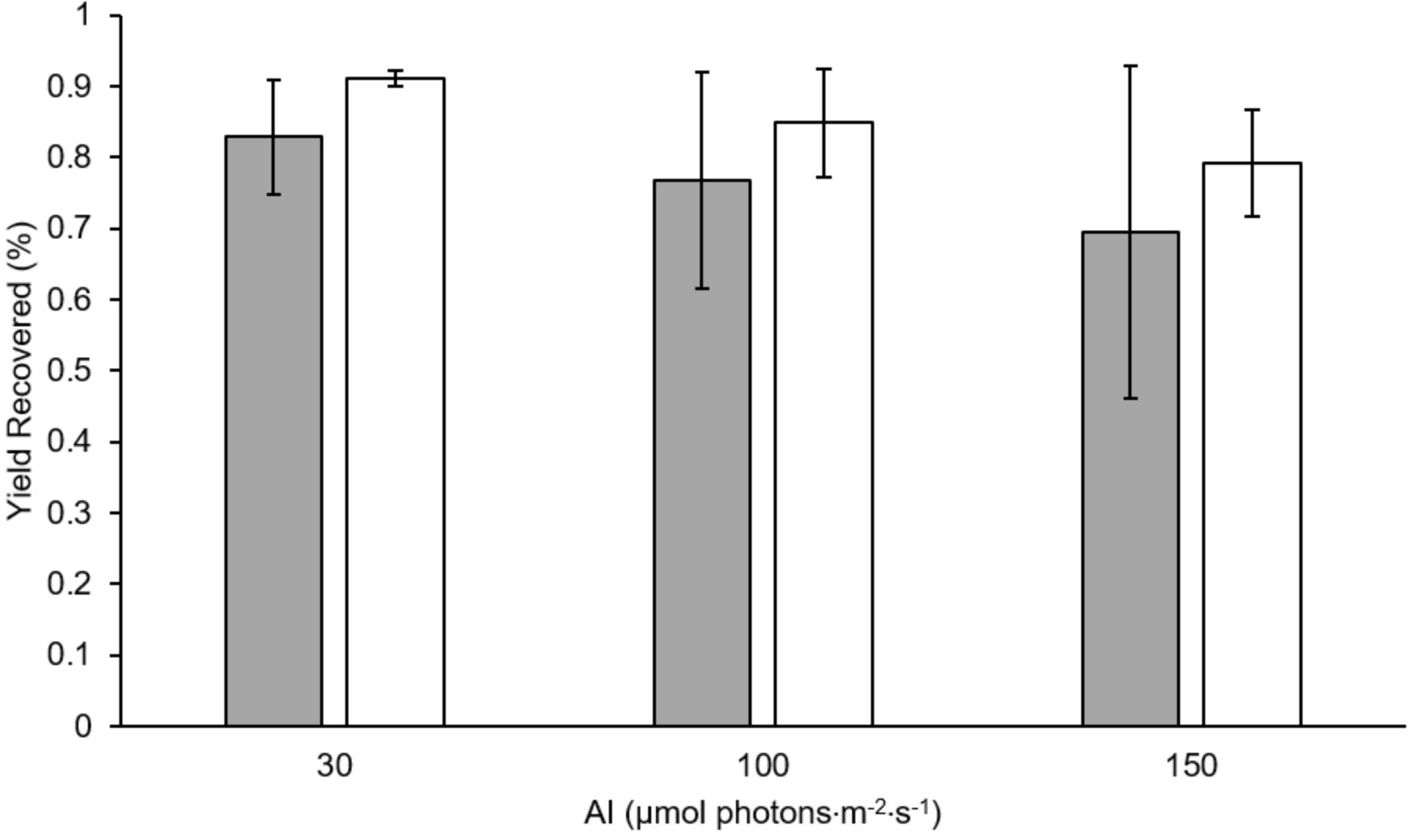

0.3
0.25
0.2
0.15
0.1
0.05
0
Growth rate (div·day-1)
0
20
40
60
80
100
120
140
160
Irradiance (µmol photons·m-2·s-1)


1.6E+06
1.4E+06
1.2E+06
1.0E+06
8.0E+05
6.0E+05
4.0E+05
2.0E+05
0.0E+00
Cell volume (µm³)
C
C
ABC
AB
BC
A*
30
100
150
AI (µmol photons·m⁻²·s⁻¹)


0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
RF·cell-1
C
C
B
C
A
BC
30
100
150
AI (µmol photons·m-2·s-1)


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Photosynthetic yield derived from P-E curves
B
A
D
C
E
D
30
100
150
AI (µmol photons·m-2·s-1)


Photosynthetic Yield
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
1400
1600
Irradiance (µmol photons·m-2·s-1)


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Yield Recovered (%)
30
100
150
AI (µmol photons·m-2·s-1)

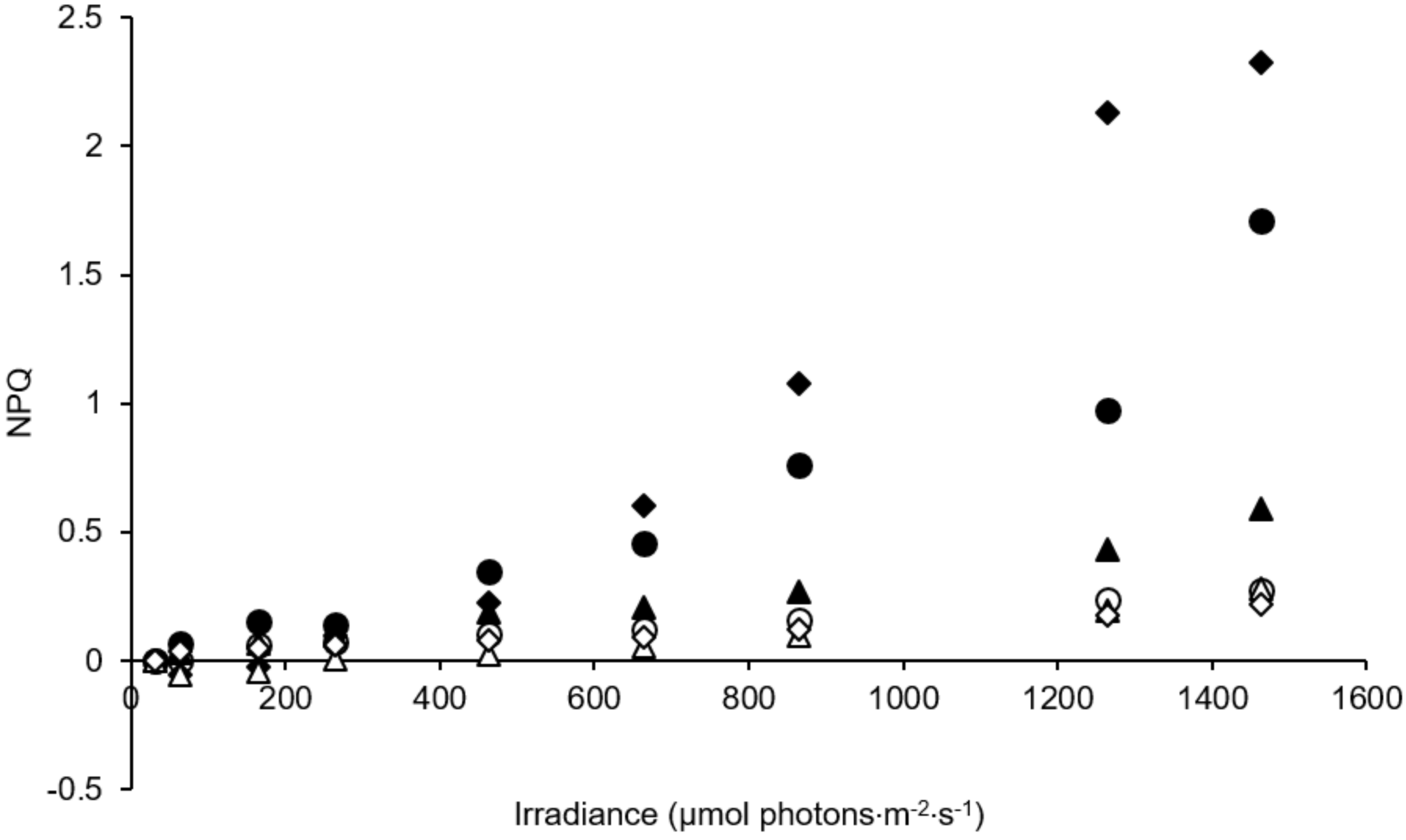

2.5
2
1.5
1
0.5
0
-0.5
NPQ
0
200
400
600
800
1000
1200
1400
1600
Irradiance (µmol photons·m-2·s-1)

Table 1: Statistical output from photosynthetic yield data analyzed using mixed-linear model. The p-values associated with specific comparisons are as follows: *G. carolinianus* low: *G. silvae* low, p = 0.032; *G. carolinianus* low: *G. silvae* optimal, p = 0.001; *G. carolinianus* optimal: *G. silvae* high, p = 0.097; all other comparisons, p = 0.000.

Species	Acclimation Irradiance	Grouped Mean Yield	Std. Error	Statistical Grouping
G. carolinianus	High	0.381	0.009	A
	Optimal	0.503	0.008	B
	Low	0.652	0.008	D
G. silvae	High	0.522	0.008	B
	Optimal	0.614	0.008	C
	Low	0.677	0.008	E