A test of the gleaner-opportunist trade-off among photosynthetic traits in Cryptophyte algae

Jake A. Swanson[1*], Matthew J. Greenwold[1,2], Tammi L. Richardson[1,3], and Jeffry L. Dudycha[1]

[1] Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

[2] Department of Biology, University of Texas at Tyler, Tyler, TX 75799, USA

[3] School of the Earth, Ocean, and Environment, University of South Carolina, Columbia, SC 29208, USA

[*] Corresponding Author: E-mail: swansoj@email.sc.edu

Open Research Statement: All data for peer review can be accessed at https://datadryad.org/stash/share/3mk8Un8vIxuhRz4vD3beLOkfhaBVlrS9UHkPfOcOpcM Upon acceptance all data will be published in the Dryad Digital Repository.

Keywords: cryptophytes, gleaner-opportunist, light intensity, photosynthesis, phylogeny, resource acquisition

Abstract

As photosynthetic producers, phytoplankton form the foundation of aquatic food webs. Understanding the relationships among photosynthetic traits in phytoplankton is essential to revealing how diversification of these traits allow phytoplankton to harvest energy from different light environments. We investigated whether the diversification of 15 species of cryptophytes, a phylum of phytoplankton with diverse light-capturing pigments, showed evidence of trade-offs among photosynthetic performance traits as predicted by the gleaner-opportunist resource exploitation framework. We constructed photosynthesis vs. irradiance (P-E) curves and rapid light curves (RLCs) to estimate parameters characterizing photosynthetic performance and electron transport rate. We inferred the evolutionary relationships among the 15 species with ultraconserved genomic elements and used a phylogenetically controlled approach to test for trade-offs. Contrary to our prediction, we observed a positive correlation between maximum photosynthetic rate, $P_{max,}$ and *P-E* α, an indicator of a species' sensitivity to increases in light intensity when light is a scarce resource. This result could not be explained by electron transfer traits, which were uncorrelated with photosynthetic rate. Together, our results suggest that ecological diversification of light exploitation in cryptophytes has escaped the constraints of a gleaner-opportunist tradeoff. Photosynthetic trade-offs may be context or scale dependent, thereby only emerging when investigated in situations different from the one used here.

Introduction

Trade-offs among traits can limit evolutionary diversification while simultaneously promoting ecological diversity (Buckling et al., 2003; Kneitel & Chase 2004; Blanchard & Moreau, 2016). Trade-offs constrain diversification by limiting the combinations of traits that are available to an organism, thereby restricting its ability to adapt to a novel environment. Conversely, trade-offs promote ecological diversity by preventing the emergence of a "Darwinian demon," i.e., an organism that is optimally adapted to its environment in all respects and therefore outcompetes all other species in its community (Kneitel & Chase, 2004). Resource allocation trade-offs have been extensively studied because they are expected to strongly influence patterns of phenotypic variation. There has been less attention to trade-offs in resource acquisition and assimilation, however, even though these processes provide the foundation for allocation decisions and can confound predictions about allocation trade-offs (Van Noordwijk & de Jong, 1986). What work has been done has largely addressed consumers (Llandres et al., 2011), which potentially overlooks the importance of resource acquisition and assimilation trade-offs for primary producers.

The gleaner-opportunist trade-off describes a trade-off between maximum growth rate and minimum resource requirement, R* (Grover, 1990; Bernhardt et al., 2020). A gleaner grows relatively well at low resource levels but relatively poorly at high resource levels (Figure 1a). In contrast, an opportunist grows relatively poorly at low resource levels but relatively well at high resource levels (Figure 1a). A gleaner, therefore, has a lower R* value for the resource while an opportunist has a higher per-capita maximum growth rate. A lower R* value indicates the ability for a population to persist at low levels of a resource, whereas a higher per-capita maximum growth rate indicates the ability to dominate a community when resources are abundant. Later,

the gleaner-opportunist framework was extended to include species-specific mortality rates (Litchman & Klauismeier, 2001).

Gleaner-opportunist trade-offs are believed to be important in the maintenance of diversity in ecological communities (Litchman et al., 2007; Yamamichi & Letten, 2022). The underlying assumption is that this trade-off allows for the coexistence of multiple species when they are competing for a variable resource. Recent work, however, has called into question the existence and relative importance of the gleaner-opportunist trade-off in structuring communities (Kiørboe & Thomas, 2020; but see Letten & Yamamichi, 2021).

Here, we envision phytoplankton as consumers of light, and apply the gleaner-opportunist framework to the photosynthetic and physiological dynamics of light capture and exploitation. We take the view that evolutionary diversification is an important structuring force in ecological communities (McPeek, 1996). Thus, our aim is to test the predictions of this resource acquisition and assimilation framework as applied to the diversification of photosynthetic traits.

Phytoplankton and photosynthesis

Phytoplankton form the foundation of most aquatic food webs (Field, 1998), thereby determining how much energy is available and influencing diversity at higher trophic levels. Phytoplankton show substantial variation of light capture and photosynthetic abilities. This includes variability of photosynthetic rates (Glover et al., 1987), of the wavelengths of light they can capture (Stomp et al., 2004), and of competitive abilities for light when light intensity fluctuates (Guislain et al., 2019). Light capture and subsequent photosynthetic performance can be considered key resource acquisition and assimilation traits (Richardson et al., 1983).

To investigate a potential gleaner-opportunist trade-off between photosynthetic traits, we performed a phylogenetically controlled analysis across a phylum of phytoplankton with diverse light capture abilities, the Cryptophyta. Cryptophytes are ideally suited to testing general predictions about photosynthetic trade-offs because they encompass substantial photosynthetic diversity, they are monophyletic, and they are commonly found in environments where competition for light may be strong. Cryptophytes' diverse light capture abilities stem from evolutionary diversification of their pigmentation (Doust et al., 2006; Greenwold et al., 2019). Cryptophyte light capture pigments include chlorophyll *a*, chlorophyll c_2, and eight phycobiliproteins that are unique to cryptophytes (Hoef-Emden & Archibald, 2016). As a taxon, cryptophytes are defined as a monophyletic group by a unique secondary endosymbiosis event (Hoef-Emden & Archibald, 2016). Most other photosynthetic taxa have limited pigment diversity (if any at all), and analyses at higher taxonomic scales are complicated by reticulate evolution. Taken together, these characteristics of cryptophytes allow for comparative investigation of photosynthetic traits while controlling for shared evolutionary history. Furthermore, as frequent inhabitants of low-light environments, cryptophytes face strong pressure to optimize photosynthetic capabilities, making them a good model for investigating adaptive hypotheses about photosynthetic diversification. Other photosynthetic organisms that live in low-light environments such as shade-tolerant trees in northern hardwood forests (Walters & Reich, 1996), understory plants in forests (Craine & Dybzinski, 2013; Onoda et al., 2015), and coral symbionts (Anthony & Hoegh-Guldberg, 2003) lack either known pigment diversity or monophyly.

We estimated cryptophyte photosynthetic parameters using photosynthesis vs. irradiance curves (P-E curves; "E" is the standard symbol for irradiance in photophysiology; Kirk, 1994)

and rapid light curves (RLCs). P-E curves provide an estimate of an organism's maximum photosynthetic rate, P_{max}, when light is abundant, along with information about how rapidly rates of photosynthesis rise with increasing light intensity at low levels (Figure 1b). The initial slope of a P-E curve, α (here referred to as *P-E* α), is the rate of photosynthesis per unit biomass per unit of incident light (Figure 1b). *P-E* α measures how effectively an organism responds to light at sub-saturating intensities (Kirk, 1994).

RLCs provide information about photosynthesis on a short time scale and provide measurements of relative electron transport rate (*rETR*), and effective quantum yield (Ralph & Gademann, 2005). Effective quantum yield is the proportion of absorbed photons that are used to drive electrons through photosystem II, whereas *rETR* is calculated by multiplying the quantum yield of photosynthesis by the photosynthetically active radiation. RLCs also provide estimates of the maximum relative electron transport rate, $rETR_{max}$, between photosystems II and I during the light-dependent reactions, and of the initial slope of a RLC (here referred to as *RLC* α) (Ralph & Gademann, 2005). *RLC* α is an estimate of the sensitivity of electron transfer to variation of light intensity at sub-saturating intensities (Ralph & Gademann, 2005) whereas $rETR_{max}$ is analogous to P_{max}; it describes the maximum rate of electron transfer between photosystem II and photosystem I under saturating light. As electron transport underpins photosynthesis more broadly, measuring electron transport traits allows us to investigate mechanisms in more detail than P-E curves.

Terminology and hypotheses

One challenge for applying the gleaner-opportunist trade-off framework to photophysiology is that the use of photosynthetic traits and terminology may cloud any

discussion. For our study, maximum photosynthetic rate and maximum electron transport will take the place of maximum growth rate in the gleaner-opportunist framework. In the photosynthesis literature, prior authors have called *P-E* α and *RLC* α "efficiency" (Kirk, 1994; Ralph & Gademann, 2005), which may cause confusion with other uses in the literature on the ecology of resource exploitation (Watt, 1986; Raubenheimer & Simpson; 1996, Tessier et al., 2000). To avoid this confusion, we will use "sensitivity" to light intensity to refer to the biological interpretation of both *P-E* α and *RLC* α. Additionally, we have physiological rather than demographic data for our species, so we define a gleaner as one that performs better at low light levels but worse at high light levels (greater *P-E* α*/RLC alpha* but lower P_{max} / $rETR_{max}$,) whereas an opportunist performs better at high light levels but worse at low light levels (lower *P-E* α*/RLC* α but higher P_{max}/$rETR_{max}$,).

We hypothesized, that if a gleaner-opportunist trade-off exists, it would manifest as a negative linear relationship between P_{max} and *P-E* α. Given that we found the opposite (see below), we hypothesized that this could be explained by a trade-off between the two parameters describing electron transport, $rETR_{max}$ and *RLC* α. Lastly, after examining both P-E curve and RLC data, we tested whether there was a positive relationship between photosynthetic rates and relative electron transport rates, predicting that greater electron transport would be correlated with a greater rate of carbon fixation.

Methods

Culture conditions

We used 15 species of cryptophytes in this experiment, 14 of which were obtained from culture repositories (Appendix S1: Section S1). We chose species to represent a wide range of

taxonomic, phylogenetic, and functional diversity. Each of the eight unique cryptophyte phycobilins are represented by at least one species in our dataset. We isolated one species from Congaree National Park, Congaree, SC, USA. Of the 15 species, 11 are identified to the species level, two to the genus level, while one, our new field isolate, is an undescribed species of *Cryptomonas* (Greenwold et al., unpublished data) Nine species are marine and six are freshwater. Additionally, we used *Goniomonas avonlea* (CCMP 3327), a non-photosynthetic cryptophyte, as an outgroup for phylogenetic estimation. More detailed culture information can be found in Appendix S1: Section S1.

Photosynthesis vs. irradiance (P-E) curves

A modified method of Lewis and Smith (1983) was used to measure photosynthesis as a function of irradiance (P-E). In short, $NaH^{14}CO_3$ was added to a 20mL sample of each species, taken when cultures were growing at mid-exponential phase, to achieve a final activity of approximately 3 μCi mL^{-1}. One mL of sample was then dispensed into each of 16 scintillation vials and vials were exposed to light intensities ranging from 0 -1400 μmol photons m^{-2} s^{-1} and irradiance was measured with a quantum scalar irradiance sensor (Biospherical Instruments, Inc., San Diego, CA, USA) inserted into an empty scintillation vial. Samples were incubated with ^{14}C for 20 minutes. After incubation, samples were terminated with 50 μL buffered formalin while dissolved inorganic carbon was driven off by adding 200 μL of 50% HCl and shaking the open vials for at least 12 h (usually overnight) in a fume hood. Five mL of scintillation cocktail (EcoLumeTM; MP Biomedicals, Solon, OH, USA) were then added to each vial, vials were mixed, and radioactive decay was counted using a Beckman LS-6500 scintillation counter (Beckman Coulter Inc., Brea, CA, USA). Photosynthetic rate for each species was measured

within a four-hour window (between 14:00 and 18:00 each day). Disintegrations per minute were then converted to chlorophyll *a*-specific primary productivity (Knap et al., 1996). Chlorophyll-specific rates of photosynthesis were plotted against light intensity and curves were fit with the equation of Platt et al. 1980 (Appendix S1: Equation S1). P_{max} and *P-E* α, were calculated following Platt et al. 1980 (Appendix S1: Equation S2). More details about the P-E curve methods can be found in Appendix S1: Section S1.

Rapid light curves and electron transport activity

Parameters of electron transport for each species were assessed via the generation of RLCs using pulse-amplitude-modulated (PAM) chlorophyll *a* fluorescence with a Walz Water-PAM (PAM, Heinz-Walz, Germany). 20mL samples of each species, again taken at mid-exponential phase, were dark-adapted for 20 minutes and then 3mL sub-samples of each species were exposed to nine pulses of actinic light increasing in intensity with a range from 30 to 1300 µmol photons $m^{-2}s^{-1}$ with a 30 second interval between each light pulse. Curves were fit like Equation S1, with P_S^B being replaced by $rETR_{mPot}$ and P^B being replaced by *rETR* (Appendix S1: Equation S3). Estimates of $rETR_{max}$ and *RLC* α were calculated in the same manner as P_{max} and *P-E* α (Appendix S1: Equation S4). Electron transport traits were measured in triplicate for each species. More details about the RLC methods can be found in Appendix S1: Section S1.

Phylogenetic estimation and comparisons across species

Any relationships among photosynthetic traits in cryptophytes may be influenced by the species' shared evolutionary history, a problem that can be avoided with phylogenetic comparative approaches (Felsenstein, 1985). Therefore, we used phylogenetic generalized least

208 squares (PGLS) to control for phylogenetic history when testing for trade-offs (Martins &
209 Hansen, 1997; Mundry, 2016). We first needed to reconstruct the evolutionary history of our
210 species, which we did with ultraconserved elements (UCEs; Faircloth et al., 2012; 2015). This
211 approach provides a genome-wide perspective on evolutionary relationships with thousands of
212 loci, providing clear benefits over work with only one or two loci (Pamilo & Nei, 1988).
213 We extracted DNA from each of our species and sent DNA samples to RAPiD
214 Genomics, LLC (Gainesville, Florida) where target enrichment sequencing was performed using
215 Illumina 2 X 150 bp reads. We used RAxML version 8.0.19 for phylogenetic inference
216 (Stamatakis, 2014) with sequence data from 1,868 conserved nuclear genome loci. Details of
217 UCE probe design, DNA extraction and sequencing, and phylogenetic inference can be found in
218 the supplementary material (Appendix S1: Section S1).
219
220 *Statistical analysis*
221 Photosynthetic and electron transport trait values were estimated with a non-linear least
222 squares method using the *minpack.lm* package (Elzhov et al., 2016).We performed PGLS
223 analyses in R version 3.6.2 (R Core Team, 2020) using the package *caper* (Orme et al., 2018).
224 We tested for correlations between P_{max} and *P-E* α and mean $rETR_{max}$ and mean *RLC* α. An
225 analysis comparing P_{max} and $rETR_{max}$ was also done post-hoc after examination of the P-E curve
226 and RLC data. PGLS models were run with habitat, phycobiliprotein absorption peak, and cell
227 volume as predictor variables. These were all non-significant predictors for all models
228 (Appendix S1: Table S3), therefore all models are shown using only the single variable of
229 interest as a predictor (Table 2). We used FigTree version 1.4.0
230 (https://github.com/rambaut/figtree/releases) to produce the phylogenetic diagram (Figure 2),

231 rooting the phylogeny with *Goniomonas avonlea* (CCMP3327). All other figures were created
232 using *ggplot2* (Wickham, 2016).
233
234 **Results**
235 *Photosynthetic parameter estimates*
236 Our estimates for P_{max} spanned two orders of magnitude, ranging from 0.071-7.10 μgC
237 $\mu Chl^{-1}\ h^{-1}$. Estimates for *P-E* α almost spanned two orders of magnitude, ranging from 0.0035-
238 0.11 μgC $\mu Chl^{-1}\ h^{-1}$ (μmol photons $m^{-2}\ s^{--1}$) (Table 1). Variation of photosynthetic parameters
239 estimated from the RLCs were constrained to less than one order of magnitude, ranging from
240 16.96-89.66 μmol electrons $m^{-2}s^{-1}$ for $rETR_{max}$ and 0.19-0.37 μmol electrons $photons^{-1}$ for *RLC* α
241 (Table 1). We also compared our estimates to photosynthetic parameter values estimated by the
242 Marine Primary Production: Model Parameters from Space (MAPPS) project, which contains
243 estimates of photosynthetic parameters from a global set of 5,711 P-E experiments with marine
244 phytoplankton (Bouman et al., 2018). Our estimates for P_{max} and *P-E* α for the 15 species of
245 cryptophytes used in this study are in the typical range of estimates from the MAPPS project,
246 indicating that cryptophytes do not have extreme photosynthetic traits (Appendix S1: Section S2,
247 Figure S2).
248
249 *Cryptophyte phylogeny*
250 We inferred a phylogeny (Figure 2) with two main clades: 1) a *Hemiselmis*/*Chroomonas*
251 clade, and 2) a *Cryptomonas*/*Rhodomonas* clade. All nodes were strongly supported. To our
252 knowledge, the phylogeny presented here is the first to apply a broad genome-wide data set to
253 Cryptophyta.

Tests of trade-offs between photosynthetic traits

P_{max} and *P-E* α are positively correlated (Figure 3a, Table 2) with the correlation having a negligible phylogenetic signal (Table 2). We found no evidence of a correlation between the maximum relative electron transport rate, $rETR_{max}$ of a species and the initial slope of its RLC, *RLC* α (Figure 3b, Table 2). There was, however, a strong phylogenetic signal (Table 2). We did not find evidence of a correlation between P_{max} and $rETR_{max}$ (Figure 3c, Table 2) however a phylogenetic signal was detected (Table 2).

Discussion

No evidence of a gleaner-opportunist trade-off

We investigated whether diversification of cryptophyte algae shows a trade-off between photosynthetic traits in the context of a gleaner-opportunist framework. We accounted for evolutionary history in our analyses, allowing us to exclude evolutionary history as a driver of the presence or absence of correlations among traits.

We found no evidence of a gleaner-opportunist trade-off between photosynthetic performance traits in cryptophytes. In fact, we found the opposite: a significant positive correlation between P_{max} and *P-E* α (Figure 3a). Cryptophytes that respond better to variation of light at low levels also perform better at high light levels, suggesting that photosynthesis is optimized simultaneously across a broad range of light intensities. All species were exposed to light intensities that ranged from 0 -1400 μmol photons m^{-2} s^{-1}, which reflect light intensity levels that marine and freshwater algae encounter in natural environments (Kirk, 1994). The intensities on the higher end of this range are sufficient to induce photoinhibition in many algae, which is when P_{max} begins to decrease as light intensity increases (Kirk, 1994). The P-E curves for our

277 species, however, do not show evidence of photoinhibition (Appendix S2). Cryptophytes are
278 usually described as low light specialists (Gervais, 1997; Hoef-Emden & Archibald, 2016) but
279 our data suggest that at least some cryptophytes photosynthesize relatively well at high light
280 levels.

281 Photosynthetic rate often correlates with population growth rate (Falkowski et al., 1985;
282 Coles & Jones, 2000) and is argued to represent the relative fitness of photosynthetic organisms
283 (Violle et al., 2007). Therefore, species with the largest values for *P-E* α and P_{max} are expected to
284 have higher average fitness in across a wide range of light intensities, compared against species
285 with lower trait values.

286 Prior work has shown that the relationship between maximum growth rate and the initial
287 slope of a functional response is strongly positively correlated with body size (Kiørboe &
288 Thomas, 2020). We accounted for this potential allometric relationship by including cell volume
289 as a fixed effect in our PGLS models and did not see a significant correlation between it and the
290 initial slope of a P-E curve or RLC. (Appendix S1: Table S3). Therefore, differences of cells'
291 volumes cannot explain the observed lack of a gleaner-opportunist trade-off.

292 There have been disagreements in the literature as to the relative importance, and even
293 existence, of the gleaner-opportunist trade-off (Kiørboe & Thomas, 2020). It has long been
294 assumed to play a crucial role in structuring ecological communities by allowing for coexistence
295 between unequal competitors. We took a novel approach to evaluate the potential origin of this
296 type of trade-off through evolutionary diversification of the traits themselves by using
297 phylogenetic comparative methods to control for the evolutionary history of our focal species. As
298 we did not find a gleaner-opportunist trade-off between photosynthetic traits our work supports

the view that this trade-off may not be as widespread as previously assumed (Litchman et al., 2007; Isanta-Navarro et al., 2022; Yamamichi & Letten, 2022).

There are, however, caveats to our results. One is that we did not incorporate species-specific mortality rates into our experiment. With the inclusion of mortality rates, an opportunist is defined by the ratio of its maximum growth rate to its mortality rate rather than simply being the species with the higher maximum growth rate when a resource is abundant (Litchman & Klausmeier, 2001). The trade-off could then potentially emerge between a species with a low resource requirement and one with a high ratio of maximum growth rate to mortality rate (Litchman & Klausmeier, 2001). In the context of our study, the ratio for defining an opportunist would be the ratio of maximum photosynthetic rate to mortality rate. By ignoring mortality rates we are potentially overlooking a condition through which a gleaner-opportunist trade-off may manifest.

No evidence of electron transport trade-offs

A possible explanation for the lack of a trade-off at the scale of overall photosynthesis is that an assimilation trade-off may exist in the electron transport chain of the light reactions but is masked by compensation in other parts of photosynthesis. This explanation is however excluded by our electron transport rate data, which showed no relationship between the initial slope of a species' RLC and its maximum relative electron transport rate (Figure 3b).

The positive relationship between *P-E* α and P_{max} could have arisen due to a mechanistic link between photosynthesis and electron transport. We expected species that were transferring more electrons between photosystems II and I to show a higher maximum photosynthetic rate as a higher rate of electron transport which would allow for more carbon to be fixed during the

Calvin Cycle (Falkowski & Raven, 2007). We tested whether maximum photosynthetic rate was positively correlated with maximum rate of electron transfer. Somewhat surprisingly, we found no evidence of a relationship (Figure 3c), indicating that greater electron transport between photosystems does not yield greater photosynthesis. This relationship, however, would really be expected only if all the energy generated by electron transport is being used to fix carbon. Physiological plasticity in diverting energy generated through electron transport to alternative metabolic pathways may explain the absence of this correlation (Halsey & Jones, 2015).

An escape from photosynthetic trade-offs?

Trade-offs promote ecological diversity by allowing competing species to coexist. Competitors may experience trade-offs in resource acquisition, resource allocation, differential predation, or dispersal ability; all these mechanisms can create the conditions necessary for stable coexistence (Chesson, 2000; Chase & Leibold, 2003; Ellner et al., 2019). Our data shows no evidence for a gleaner-opportunist trade-off between photosynthetic traits in cryptophytes. Thus, some cryptophytes should be strong competitors across a wide range of light environments. This lack of a trade-off, specifically between P_{max} and *P-E* α, has been observed before and physiological mechanisms have been suggested as the cause of this relationship (Behrenfeld et al., 2008; Halsey et al., 2010).

In ecological comparisons of phytoplankton growing in different nutrient environments, Halsey et al. (2011; 2013; 2014) suggested positive covariation between P_{max} and *P-E* α is driven by carbon metabolism occurring via different pathways. These researchers manipulated nitrogen or light to limit growth rates in green algae and diatoms, thereby producing positive covariation of P_{max} and *P-E* α across environments due to changes in carbon metabolism. They argue that at

low growth rates, induced by nitrogen or light limitation, a fixed transient carbon pool is quickly used for synthesis of ATP or NADPH, nucleic acids, and lipids. In contrast, at high growth rates fixed transient carbon is mostly stored as polysaccharides and used over a longer timescale. Thus, these within-species environmental manipulations recovered the same pattern as our across-species evaluation of evolutionary diversification, but it is unclear whether the proposed mechanism could be the same. First, in our study, all cultures were grown in nutrient-rich media for short periods of time, and thus none should have been nitrogen-limited. This is particularly true because our measurements were taken at mid-exponential phase. Second, we do not have information on the transient carbon pool or polysaccharide storage to characterize carbon metabolism in cryptophytes. Knowing a positive relationship exists, acquiring these types of data becomes a priority for future work.

The carbon metabolism hypothesis provides a potential explanation for the observed lack of gleaner-opportunist and power-efficiency trade-offs but does not rule out the possibility that trade-offs occur between other resource acquisition traits that we did not investigate. In fact, its empirical link to nitrogen limitation points to the importance of considering different types of resources. Trade-offs among resource acquisition traits in phytoplankton are well known, such as being a strong competitor for light but a weak competitor for nutrients (Tilman, 1977; Litchman & Klausmeier, 2008). Phytoplankton resource acquisition traits are remarkably plastic (Stomp et al., 2008; Hattich et al., 2016) so environmental variability such as intermittent predation pressure, sporadic nutrient limitation, interspecific competition, or temperature shifts could potentially drive the emergence of photosynthetic trade-offs. Over evolutionary timescales, cryptophytes that display low trait values for both P_{max} and P-E α may have optimized other

aspects of fitness to be excellent competitors for other resources, leading to trade-offs among traits not investigated in this study.

Implications for natural phytoplankton communities

While our study aimed to broadly examine photosynthetic trade-offs through the gleaner-opportunist framework, our results also imply that the niches of cryptophytes in natural phytoplankton communities may be misunderstood. In particular, the realized niche of cryptophytes may have historically been interpreted as their fundamental niche. Cryptophytes are often described as low-light specialists (Hoef-Emden & Archibald, 2016) and frequently occur at deeper depths where less light is available (Gervais, 1997). At these depths, light attenuation leads to a decrease in the number of photons available for photosynthesis and alters the color of available light. Absorption by chlorophyll *a* and colored dissolved organic matter reduces the blue light present in the environment while green and red light are absorbed by accessory pigments like phycoerythrin and phycocyanin. In conjunction with the MAPPS data (Appendix S1: Figure S2), our results suggest that cryptophytes are not exceptional at capturing photons at low light intensities when compared to other taxa of phytoplankton, in contrast to expectations for low-light specialists. Furthermore, our P-E curves (Appendix S2) provide no evidence for photoinhibition at high light intensities. Cryptophytes instead have a broad fundamental niche with respect to light intensity and are better thought of as low-light tolerant rather than specialists. Cryptophytes may use their unique pigmentation to absorb colors of light that are not absorbed by taxa present at the surface. They could be exploiting fine gradations of light color rather than light intensity to carve out realized niches in different aquatic ecosystems. In addition to being able to exploit different colors of light, cryptophytes may succeed at deeper depths

because due to greater nutrient availability than surface waters. Cryptophytes require nitrogen for phycobiliprotein synthesis (Doust et al., 2006) and have been shown to rapidly degrade these nitrogen-containing pigments when nitrogen is scarce (Da Silva et al., 2009). Living deeper may provide cryptophytes with access to sufficient nitrogen to synthesize phycobiliproteins, which then allows them to exploit wavelengths of light for photosynthesis that are not absorbed by other taxa.

Future directions for trade-off research

Trade-offs may be dependent on the scale at which they are investigated (i.e., at the level of phenotype, genotype, population, or species) (Agrawal, 2020). For example, a relationship may be seen from a within-species comparison, but the same relationship may not manifest at the among-species level. This pattern of trade-offs occurring across different scales can be seen in trade-offs between milkweed leaf traits (Agrawal, 2020). A trade-off between leaf mass per area and cardenolide concentration can be seen between populations but is not apparent at the level of genotype or species (Agrawal, 2020). For plant traits, the general expectation is that trade-offs or predicted trade-offs will not be persistent across different scales (Agrawal, 2020). This paradigm of scale-dependent trade-offs may also be applicable to phytoplankton and may provide an investigative framework for future work on photosynthetic trait trade-offs.

Our study tested for photosynthetic trade-offs within an explicit resource exploitation framework while controlling for evolutionary history via phylogenetic comparative methods. This approach should be applicable for researchers working in systems where competition for light among photosynthetic organisms plays a strong role in structuring communities. This may include aquatic macrophytes (Sand-Jensen et al., 2007), forest communities (Onoda et al., 2015),

413 grassland communities (Dybzinski & Tilman, 2007; Hautier et al., 2009), and coral
414 endosymbionts (McIlroy et al., 2019). Examining strategies for light capture through pre-existing
415 ecological frameworks can provide new perspectives and insights on how photosynthetic
416 organisms interact with light in their environment.
417

418 **Acknowledgments**

419 We thank Kristin Heidenreich, Krista Harmon, Cameron Riddick, and Patrick Lawson for
420 help with culture maintenance and Jay Pinckney for the inspiration and equipment for measuring
421 electron transport rate via rapid light curves. This study was supported by the National Science
422 Foundation (NSF) Dimensions of Biodiversity program under grant #1542555 to T.L.
423 Richardson and J. L. Dudycha.
424

425 **Author Contributions**

426 The experiment was designed by JAS, TLR, and JLD. Data for the P-E curves and RLCs
427 were collected by JAS. DNA extractions and phylogeny creation was done by MJG. Statistical
428 analyses were done by JAS. The manuscript was written by JAS, MJG, and JLD with all authors
429 contributing to the editing process.
430

431 **Conflicts of Interest**

432 The authors declare no conflicts of interest.

References

Anthony, K.R.N., and O. Hoegh-Guldberg. 2003. "Variation in Coral Photosynthesis, Respiration and Growth Characteristics in Contrasting Light Microhabitats: An Analogue to Plants in Forest Gaps and Understoreys?". *Functional Ecology* 17: 246-259.

Behrenfeld, M.J., K.H. Halsey, and A.J. Milligan. 2008. "Evolved Physiological Responses of Phytoplankton to their Integrated Growth Environment". *Philosophical Transactions of the Royal Society B* 363: 2687-2703.

Bernhardt, J.R., P. Kratina, A.L., Pereira, M. Tamminen, M.K., Thomas, and A. Narwani 2020. "The Evolution of Competitive Ability for Essential Resources". *Philosophical Transactions of the Royal Society B* 375: 20190247.

Blanchard, B. D., and C.S. Moreau. 2017. "Defensive Traits Exhibit an Evolutionary Trade-Off and Drive Diversification in Ants". *Evolution* 71: 315–328.

Bouman, H. A., T. Platt, M. Doblin, F.G. Figueiras, K. Gudmundsson, H.G. Gudfinnsson, B. Huang, et al. 2018. "Photosynthesis–Irradiance Parameters of Marine Phytoplankton: Synthesis of a Global Data Set". *Earth System Science Data* 10: 251–266.

Buckling, A. 2003. "Adaptation Limits Diversification of Experimental Bacterial Populations". *Science* 302: 2107–2109.

Chase, J. M., and M.A. Leibold. 2003. *Ecological Niches: Linking Classical and Contemporary Approaches*. Chicago, IL: University of Chicago Press.

Chesson, P. 2000. "Mechanisms of Maintenance of Species Diversity". *Annual Review of Ecology and Systematics* 31: 43-366.

Craine, J.M., and R. Dybzinski. 2013. "Mechanisms of Plant Competition for Nutrients, Water, and Light". *Functional Ecology* 27: 833-840.

456 Doust, A. B., K.E. Wilk, P.M.G. Curmi, and G.D. Scholes. 2006. "The Photophysics of
457 Cryptophyte Light-Harvesting". *Journal of Photochemistry and Photobiology, A* 184: 1–
458 17.
459 Dybzinski, R., and D. Tilman. 2007. “Resource Use Patterns Predict Long-Term Outcomes of
460 Plant Competition for Nutrients and Light”. *The American Naturalist* 170: 305-318.
461 Ellner, S. P., R.E. Snyder, P.B Adler, and G. Hooker. 2019. "An Expanded Modern Coexistence
462 Theory for Empirical Applications". *Ecology Letters* 22: 3-18.
463 Elzhov, T.V, K.M, Mullen, A.N. Speiss, and B. Bolker. 2016. "minpack.lm: R Interface to the
464 Levenberg-Marquardt Nonlinear Least-Squares Algorithm Found in MINPACK, Plus
465 Support for Bounds. R package version 1.2-1".
466 https://CRAN.Rproject.org/package=minpack.lm
467 Faircloth, B. C., M.G. Branstetter, N.D. White, and S.G. Brady. 2015. "Target Enrichment of
468 Ultraconserved Elements from Arthropods Provides a Genomic Perspective on
469 Relationships among Hymenoptera". *Molecular Ecology Resources* 15: 489-501.
470 Faircloth B.C., J.E. McCormack, N.G Crawford, M.G. Harvey, R.T. Brumfield, and T.C. Glenn.
471 2012. "Ultraconserved Elements Anchor Thousands of Genetic Markers Spanning
472 Multiple Evolutionary Timescales". *Systematic Biology* 61: 717-726.
473 Felsenstein, J. 1985. "Phylogenies and the Comparative Method". *The American Naturalist* 125:
474 1–15.
475 Field, C. B. 1998. "Primary Production of the Biosphere: Integrating Terrestrial and Oceanic
476 Components". *Science* 281: 237–240.
477 Gervais, F. 1997. "Light-Dependent Growth, Dark Survival, and Glucose Uptake by
478 Cryptophytes Isolated from a Freshwater Chemocline". *Journal of Phycology* 33: 18–25.

479 Glover, H. E., M.D. Keller, and R.W. Spinrad. 1987. "The Effects of Light Quality and Intensity
480 on Photosynthesis and Growth of Marine Eukaryotic and Prokaryotic Phytoplankton
481 Clones". *Journal of Experimental Marine Biology and Ecology* 105: 137–159.
482 Greenwold, M. J., B.R. Cunningham, E.M. Lachenmyer, J.M. Pullman, T.L.Richardson, and J.L.
483 Dudycha. 2019. "Diversification of Light Capture Ability was Accompanied by the
484 Evolution of Phycobiliproteins in Cryptophyte Algae". *Proceedings of the Royal Society*
485 *B* 286: 20190655.
486 Grover, J. P. 1990. "Resource Competition in a Variable Environment: Phytoplankton Growing
487 According to Monod's Model". *The American Naturalist* 136: 771–789.
488 Guislain, A., B.E. Beisner, and J. Köhler. 2019. "Variation in Species Light Acquisition Traits
489 Under Fluctuating Light Regimes: Implications for Non-Equilibrium Coexistence". *Oikos*
490 128: 716–728.
491 Halsey, K. H., and B.M. Jones. 2015. "Phytoplankton Strategies for Photosynthetic Energy
492 Allocation". *Annual Review of Marine Science* 7: 265–297.
493 Halsey, K.H., A.J. Milligan, and M.J. Behrenfeld. 2010. "Physiological Optimization Underlies
494 Growth Rate-Independent Chlorophyll-Specific Gross and Net Primary Production".
495 *Photosynthesis Research* 103: 125–137.
496 Halsey, K.H., A.J. Milligan, and M.J. Behrenfeld. 2011. "Linking Time-Dependent Carbon-
497 Fixation Efficiencies in *Dunaliella Tertiolecta* (Chlorophyceae) to Underlying Metabolic
498 Pathways". *Journal of Phycology* 47: 66-76.
499 Halsey, K.H., R.T. O'Malley, J.R. Graff, A.J. Milligan, and M.J. Behrenfeld. 2013. "A Common
500 Partitioning Strategy for Photosynthetic Products in Evolutionarily Distinct
501 Phytoplankton Species". *New Phytologist* 198: 1030-1038.

Hattich, G. S. I., L. Listmann, J. Raab, D. Ozod-Seradj, T.B.H. Reusch, and B. Matthiessen. 2017. "Inter- and Intraspecific Phenotypic Plasticity of Three Phytoplankton Species in Response to Ocean Acidification". *Biology Letters* 13: 20160774.

Hautier, Y., P.A. Niklaus, and A. Hector. 2009. “Competition for Light Causes Plant Biodiversity Loss After Eutrophication”. *Science* 324: 636-638.

Hoef-Emden, K., and J.M. Archibald. 2016. "Cryptophyta (Cryptomonads)". In *Handbook of the Protists*, edited by J.M. Archibald, A.G.B. Simpson, C.H. Slamovits, L. Margulis, M. Melkonian, D.J. Chapman, and J.O. Corliss, 1-41. Springer International Publishing.

Isanta-Navarro, J., T. Klauschies, A. Wacker, and D. Martin-Creuzburg. 2022. "A Sterol-Mediated Gleaner–Opportunist Trade-Off Underlies the Evolution of Grazer Resistance to Cyanobacteria". *Proceedings of the Royal Society B* 289: 20220178.

Kiørboe, T., and M.K. Thomas. 2020. "Heterotrophic Eukaryotes Show a Slow-Fast Continuum, Not a Gleaner–Exploiter Trade-Off". *Proceedings of the National Academy of Sciences* 117: 24893-24899.

Kirk, J. T. O. 1994. *Light & Photosynthesis in Aquatic Ecosytems*. Cambridge, UK: Cambridge University Press.

Knap, A., A. Michaels, A. Close, H.W. Ducklow, and H. Dickson. 1996. "Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements. Report no. 19, Reprint from the IOC Manuals and Guides no. 29". Bergen: UNESCO.

Kneitel, J. M., and J.M. Chase. 2004. "Trade-Offs in Community Ecology: Linking Spatial Scales and Species Coexistence". *Ecology Letters* 7: 69–80.

523 Letten, A.D., and M. Yamamichi. 2021. "Gleaning, Fast and Slow: In Defense of a Canonical
524 Ecological Trade-Off". *Proceedings of the National Academy of Sciences,* 118:
525 e2022754118.
526 Lewis, M., and J. Smith. 1983. "A Small Volume, Short-Incubation-Time Method for
527 Measurement of Photosynthesis as a Function of Incident Irradiance". *Marine Ecology*
528 *Progress Series* 13: 99–102.
529 Litchman, E., and C.A. Klausmeier. 2001. "Competition of Phytoplankton Under Fluctuating
530 Light". *The American Naturalist* 157: 170–187.
531 Litchman, E., C.A. Klausmeier, O.M. Schofield, and P.G. Falkowski. 2007. "The Role of
532 Functional Traits and Trade-Offs in Structuring Phytoplankton Communities: Scaling
533 from Cellular to Ecosystem Level". *Ecology Letters* 10: 1170-1181.
534 Llandres, A. L., E. De Mas, and M.A. Rodríguez-Gironés. 2012. "Response of Pollinators to the
535 Tradeoff between Resource Acquisition and Predator Avoidance". *Oikos* 121: 687–696.
536 Martins, E.P., and T.F. Hansen. 1997. "A General Approach to Incorporating Phylogenetic
537 Information into the Analysis of Interspecific Data". *The American Naturalist* 149: 646-
538 667.
539 McIlroy, S.E., R. Cunning, A.C. Baker, and M.A. Coffroth. 2019. “Competition and Succession
540 Among Coral Endosymbionts”. *Ecology and Evolution* 9: 12767-12778.
541 McPeek, M. A. 1996. "Linking Local Species Interactions to Rates of Speciation in
542 Communities". *Ecology* 77: 1355-1366.
543 Mundry, R. 2014. "Statistical Issues and Assumptions of Phylogenetic Generalized Least
544 Squares". In *Modern Phylogenetic Comparative Methods and theirAapplication in*

Evolutionary Biology, edited by L.Z. Garamszegi, 131-153. Springer-Verlag Berlin Heidelberg.

van Noordwijk, A. J., and G. de Jong. 1986. "Acquisition and Allocation of Resources: Their Influence on Variation in Life History Tactics". *The American Naturalist* 128: 137–142.

Onoda, Y., J.B. Saluñga, K. Akutsu, S. Aiba, T. Yahara., and N.P.R. Anten. 2014. "Trade-off between Light Interception Efficiency and Light Use Efficiency: Implications for Species Coexistence in One-Sided Light Competition". *Journal of Ecology* 102: 167-175.

Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2018. "caper: Comparative Analyses of Phylogenetics and Evolution in R. R Package Version 1.0.1". https://CRAN.R-project.org/package=caper

Pamilo, P., and M. Nei. 1988. "Relationships between Gene Trees and Species Trees". *Molecular Biology and Evolution* 5: 568-583.

Platt, T., C.L. Gallegos, and W.G. Harrison. 1980. "Photoinhibition of Photosynthesis in Natural Assemblage of Marine Phytoplankton". *Journal of Marine Research* 38: 687-701.

Ralph, P. J., and R. Gademann. 2005. Rapid Light Curves: A Powerful Tool to Assess Photosynthetic Activity. *Aquatic Botany* 82: 222–237.

Raubenheimer, D., and S.J. Simpson. 1996. "Meeting Nutrient Requirements: The Roles of Power and Efficiency". *Entomologia Experimentalis et Applicata, 80*, 65–68.

Richardson, K., J. Beardall, and J.A. Raven. 1983. Adaptation of Unicellular Algae to Irradiance: an Analysis of Strategies. *New Phytologist* 93: 157–191.

R Core Team 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Sand-Jensen, K., T. Binzer, and A.L. Middelboe. 2007. "Scaling of Photosynthetic Production of

568 Aquatic Macrophytes – A Review". *Oikos* 116: 280-294.

569 da Silva, A. F., S.O. Lourenço, and R.M. Chaloub. 2009. Effects of Nitrogen Starvation on the
570 Photosynthetic Physiology of a Tropical Marine Microalga *Rhodomonas* sp.
571 (Cryptophyceae). *Aquatic Botany* 91: 291–297.

572 Stamatakis, A. 2014. "RAxML version 8: a tool for phylogenetic analysis and post-analysis of
573 large phylogenies". *Bioinformatics* 30: 1312-1313.

574 Stomp, M., M.A. van Dijk, H.M.J. van Overzee, M.T. Wortel, C.A.M. Sigon, M. Egas, H.
575 Hoogveld, H.J. Gons, and J. Huisman. 2008. "The Timescale of Phenotypic Plasticity and
576 its Impact on Competition in Fluctuating Environments". *The American Naturalist* 172:
577 E169–E185.

578 Stomp, M., J. Huisman, D. Gerla, M. Rijkeboer, B.W. Ibelings, U.I.A. Wollenzien, and L.J. Stal.
579 2004. "Adaptive Divergence in Pigment Composition Promotes Phytoplankton
580 Biodiversity". *Nature* 432: 104-107.

581 Tessier, A. J., M.A. Leibold, and J. Tsao. 2000. "A Fundamental Trade-Off in Resource
582 Exploitation by *Daphnia* and Consequences to Plankton Communities". *Ecology* 81:
583 826–841.

584 Tilman, D. 1977. "Resource Competition between Plankton Algae: An Experimental and
585 Theoretical Approach". *Ecology* 58: 338–348.

586 Violle, C., M.L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. "Let
587 the Concept of Trait Be Functional!" *Oikos* 116: 882–892.

588 Walters, M.B., and P.B. Reich. 1996. "Are Shade Tolerance, Survival, and Growth Linked? Low
589 Light and Nitrogen Effects on Hardwood Seedlings". *Ecology* 77: 841-853.

590 Watt, W. B. 1986. "Power and Efficiency as Indexes of Fitness in Metabolic Organization". *The*
591 *American Naturalist* 127: 629–653.
592 Wickham, H. 2016. "ggplot2: elegant graphics for data analysis". Springer-Verlag New
593 York.
594 Yamamichi, M., and A.D. Letten. 2021. "Rapid Evolution Promotes Fluctuation-Dependent
595 Species Coexistence". *Ecology Letters* 24: 812-818.
596 Yamamichi, M., and A.D. Letten. 2022. "Extending the Gleaner-Opportunist Trade-Off".
597 *Journal of Animal Ecology* 00:1-8.
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610 **Table 1:** Values for photosynthetic performance traits and electron transport rate traits for each

611 cryptophyte species

Species	*P-E* α (µgC µChl a^{-1} h^{-1} (µmol photons m^{-2} $s^{-1})^{-1}$)	P_{max} (µgC µChl a^{-1} h^{-1})	*RLC* α (µmol electrons photons^{-1})	$rETR_{max}$ (µmol electrons m^{-2} s^{-1})
Cryptomonas ovata	0.030	3.07	0.323	58.86
Chroomonas mesostigmatica	0.065	2.84	0.205	21.28
Chroomonas nordstedtii	0.0035	0.072	0.297	35.86
Chroomonas placoidea	0.023	2.67	0.194	21.83
Chroomonas sp.	0.015	0.24	0.222	20.78
Cryptomonas sp.	0.048	1.93	-	-
Guillardia theta	0.024	3.19	0.301	67.06
Hemiselmis cryptochromatica	0.11	2.66	0.256	27.06
Hemiselmis pacifica	0.031	1.79	0.356	16.96
Hemiselmis tepida	0.030	1.43	0.212	19.04
Proteomonas sulcata	0.058	3.05	0.367	29.31
Rhodomonas minuta	0.061	3.47	0.323	89.66
Rhodomonas salina	0.018	1.56	0.374	30.29
Teleaulax sp.	0.039	2.24	0.374	77.74
Unid. sp.	0.10	7.10	0.328	35.89

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Table 2: Summary of results from PGLS regression models. Significant *p*-values are in bold.

PGLS Model	Intercept	*F*	Df	Adjusted R^2	*p*	N	Pagel's λ
P_{max} ~ P-E_alpha	0.88	12.12	1,13	0.44	**0.0041**	15	0.054
$rETR_{max}$ ~ RLC_alpha	20.66	0.53	1,12	-0.038	0.48	14	1
P_{max} ~ rETRmax	2.64	0.038	1,12	-0.080	0.85	14	1

Figure 1: a) Visualization of the gleaner-opportunist framework, adapted from Litchman & Klausmeier, 2001. A gleaner (blue line) has a higher per-capita growth rate at low resource concentrations while an opportunist (gold line) has a higher per-capita growth when resources are abundant. b) Example photosynthesis vs. irradiance curve for the cryptophyte *Rhodomonas salina* indicating the initial slope of the P-E curve, *P-E* α, and the maximum photosynthetic rate, P_{max}

Figure 2: Phylogeny for the 15 cryptophyte species constructed using ultra-conserved elements. Bootstrap values shown at each node.

Figure 3: Relationships between photosynthetic parameters derived from P-E curves and RLC. a) Maximum photosynthetic rate, P_{max} (μg C (μ chl *a*)$^{-1}$ h^{-1}), of a species and *P-E* α (μg C (μ chl *a*)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$, the initial slope of its P-E curve. b) Maximum relative electron transport rate, $rETR_{max}$ (μmol electrons m^{-2}s^{-1}) and the initial slope of a RLC, *RLC* α (electrons photons^{-1}). Points are the mean values of triplicate estimates for $rETR_{max}$ and *RLC* α for each species or species. c) No correlation between the maximum photosynthetic rate of a species, P_{max} (μg C (μ chl *a*)$^{-1}$ h^{-1}), and its maximum relative electron transport rate, $rETR_{max}$ (μmol electrons m^{-2}s^{-1}). *rETRmax* values represent the mean of triplicate estimates for each species or species.

Figure 1

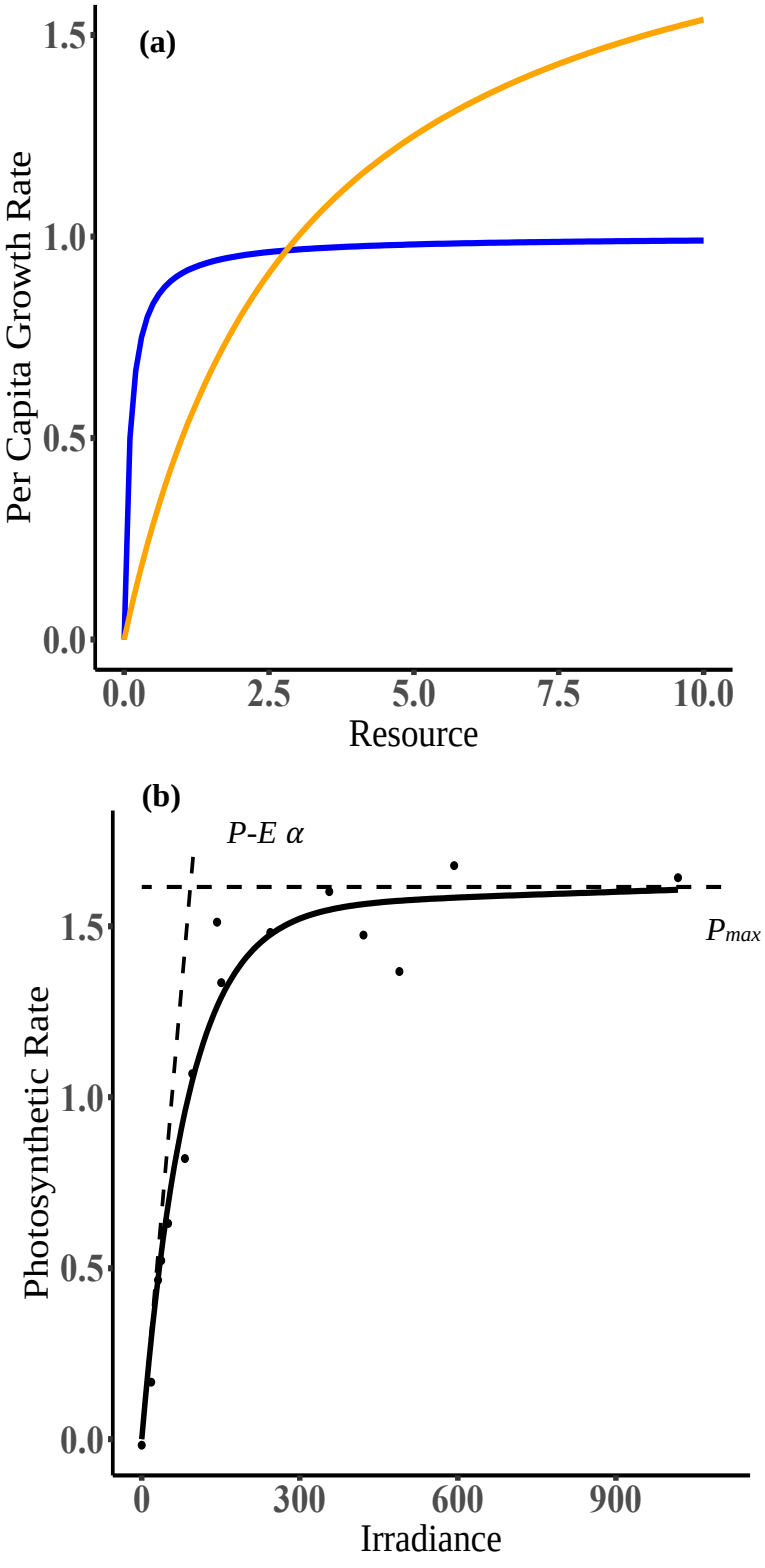

Figure 2

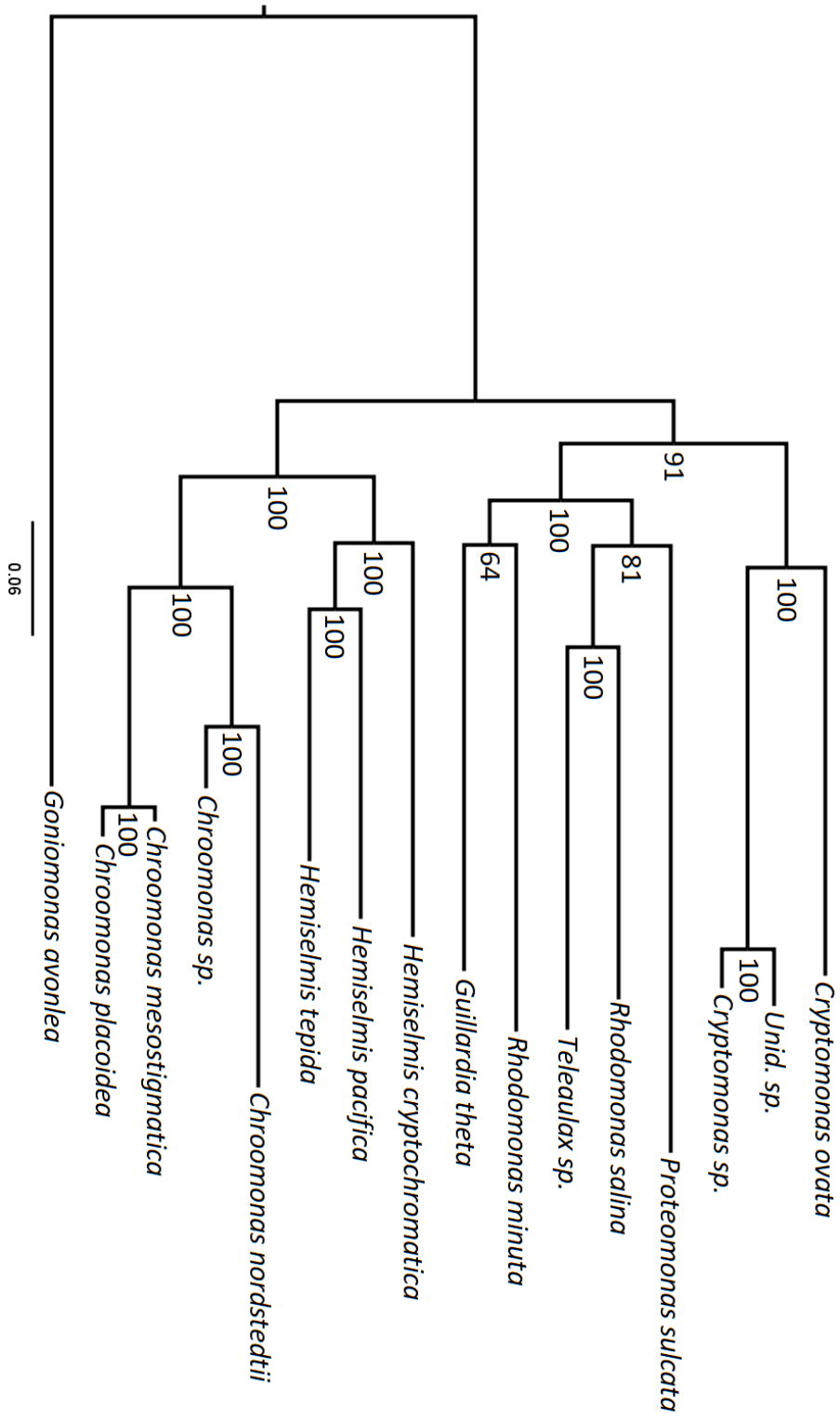

Figure 3