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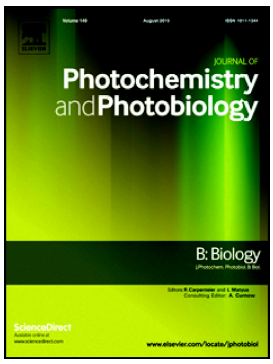

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Implications of irradiance exposure and non-photochemical quenching for multi-wavelength (bbe FluoroProbe) fluorometry

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ABSTRACT

Multi-wavelength fluorometers, such as the bbe FluoroProbe (FP), measure excitation spectra of chlorophyll a (Chl-a) fluorescence to infer the abundance and composition of phytoplankton communities as well as the concentration of chromophoric dissolved organic matter (CDOM). Experiments were conducted on laboratory cultures and on natural communities of freshwater phytoplankton to determine how the response of phytoplankton to high irradiance might affect fluorometric estimates of community composition and concentrations of Chl-a and CDOM. Cultures of a representative cyanobacterium, bacillariophyte, synurophyte, cryptophyte, and chlorophyte revealed changes in Chl-a excitation spectra as irradiance was increased to saturating levels and non-photochemical quenching (NPQ) increased. The degree of change and resulting classification error varied among taxa, being strong for the synurophyte and cryptophyte but minimal for the cyanobacterium. Acute-exposure experiments on phytoplankton communities of varying taxonomic composition from five lakes yielded variable results on apparent community composition. There was a consistent decrease in CDOM estimates, whereas Chl-a estimates were generally increased. Subsequent exposure to low PAR relaxed NPQ and tended to reverse the effects of high irradiance on composition, total Chl-a, and CDOM estimates. Relaxation experiments on near-surface communities in a sixth, large lake, Georgian Bay, showed that total Chl-a estimates increased by 44% on average when dark treatments were used to relax NPQ, though, in contrast to the findings from the small lakes, there was little effect on CDOM estimates. We observed a statistically-significant, negative linear relationship between the photon flux density of *in situ* irradiance and the accuracy of taxonomic assignment by FP in Georgian Bay. Not discounting the correlations between light intensity and the accuracy of the FP that were observed in this study, we conclude that the applicability of the

reference spectra to the system under investigation is a more important consideration than variability in natural irradiance conditions.

Keywords: FluoroProbe, spectral fluorescence, phytoplankton, NPQ, CDOM

INTRODUCTION

The measurement of chlorophyll a (Chl-a) fluorescence emission provides a rapid and sensitive proxy for phytoplankton biomass, and its utility in quantifying phytoplankton communities in nature has been recognized for over half a century [1]. Phytoplankton groups of differing photosynthetic pigmentation can be distinguished based on fluorescence excitation spectra [2, 3]. Submersible spectral fluorometers, such as the FluoroProbe (FP; bbe Moldaenke GmbH, Kiel, Germany) can be used to quantify phytoplankton community composition across large scales for which sample collection and examination via microscopy would not be possible. The FP measures fluorescence emission at ~680 nm in response to excitation by light emitting diodes (LEDs) centered at approximately 370, 470, 525, 570, 590, and 610 nm. The accompanying software estimates the Chl-a of different phytoplankton pigment groups as well as chromophoric dissolved organic matter (CDOM) concentrations (i.e., 'yellow substances'), by de-convoluting (or "unmixing", [4]) the observed spectrum based on reference spectra that are established through calibration with algal cultures and CDOM standards [5]. We use the terminology of Catherine *et al*. ([6]) for different spectral groups and the (distinctive) pigments and taxa that they represent: *green* (Chl-b; Chlorophyta and Euglenophyta), *brown* (Chl-c and fuxoxanthin/peridinin; Heterokontophyta (e.g., Bacillariophyceae, Synurophyceae, and Chrysophyceae) and Pyrrhophyta), *blue* (phycocyanin (PC); PC-rich Cyanobacteria), and *red* (phycoerythrin (PE); Cryptophyta and PE-rich Cyanobacteria). These groups (green, brown, blue, and red) correspond to what the FP software resports as Green Algae, Bluegreen, Diatoms, and Cryptophyta, respectively. For accurate results, it is important that reference spectra truly represent the groups actually present, and both physiological and taxonomic variation can make this assumption hard to satisfy [4, 7]. There has nonetheless been extensive validation of FP, and

its bench-top counterpart (Algae Online Analyzer, AOA), against independent measures of Chl-a and phytoplankton taxonomy (e.g., [6, 8-10]). These instruments have been proven to make useful estimates, but considerable residual variation often remains, and systematic errors in total and group-specific abundance estimates have been documented for both the FP [6, 7, 11] and AOA [9, 12]. The error can have many sources, not all of which can be assumed to reside in the FP methodology, but non-photochemical quenching is a likely contributor in many cases [13].

It was noted shortly after its advent within oceanography that the interpretation of *in situ* Chl-a fluorescence is complicated by apparent variability in the fluorescence yield of Chl-a contained within living cells, part of which is due to solar radiation [14]. Primary producers utilize a variety of mechanisms to protect Photosystem II (PSII) from the photodamage that can occur when the supply of quanta to PSII exceeds demand [15, 16]. The operation of these mechanisms manifests as an altered fluorescence yield of Chl-a [17-19]. The altered yields may be expected to affect not only total Chl-a estimates but also estimates of the abundance of individual pigment groups as obtained from commercially-available multi-wavelength fluorometers such as the FP. Fluorometric estimates of CDOM could also be affected by sunlight-induced changes in the CDOM fluorescence excitation spectrum (e.g., [20]), and might be indirectly affected by changes in phytoplankton excitation spectra. As use of multi-wavelength fluorometers increases (e.g., [21-23]), there is a need to better understand how phytoplankton responses to high irradiance may affect such measurements.

Under high irradiance exposure, thermal dissipation of excitation energy or the reduction of exciton delivery to PSII are observable as a decrease in PSII fluorescence known as nonphotochemical quenching (NPQ; see [24] for a recent and comprehensive review). NPQ is commonly assessed using pulse amplitude modulated (PAM) fluorometry [25]. An important

NPQ mechanism in most eukaryotic phytoplankton is the xanthophyll cycle: the reversible de-epoxidation of xanthophyll pigments (oxygenated carotenoids) in response to high light [26], which protects PSII from over-excitation by dissipating excitation energy as heat [27]. Another mechanism, state transitions [28, 29], involve adjustment of the relative sizes of the light harvesting complexes (LHCs) associated with PSII and Photosystem I (PSI), and are triggered by the redox status of the plastoquinone (PQ) pool and the NADPH/ATP ratio ([16] and references therein).

The capacity for NPQ and its underlying mechanisms vary taxonomically, with potential differences in fluorescence quenching among pigment groups. The diadinoxanthin-diatoxanthin (Dd-Dt) xanthophyll cycle is common in many diatoms [30], as well as some other chlorophyll-c containing phytoplankton (e.g., chrysophytes, dinoflagellates, xanthophytes; [17]). Chlorophytes use the violaxanthin-antheraxanthin-zeaxanthin (VAZ) cycle, and, to a lesser extent, state transitions [31]. Cyanobacteria accomplish NPQ by state transitions, blue-green-light induced thermal dissipation mediated by the orange carotenoid protein, and phycobilisome decoupling [19]. NPQ in cryptophytes is less well studied, though they have been shown to use "energy-dependent" quenching (qE) in the Chl-a/c antennae [32], and at least one species (*Guillardia theta*) has been reported to undergo state transitions [33].

If some phytoplankton taxa exhibit more NPQ than others, or if the shape of any taxon's excitation spectrum is modified by exposure to high light [4], we may expect effects on FP estimates of both total Chl-a concentration and community composition. Indeed, the potential changes in Chl-a fluorescence intensity of different taxa due to NPQ can vary appreciably, with the underlying response mechanisms affecting the magnitude and duration of fluorescence quenching [24]. CDOM estimates can be affected directly if sunlight exposure alters the CDOM

excitation spectrum [34], while both CDOM and Chl-a estimates could be affected indirectly if altered excitation spectra for the algae and cyanobacteria cause the instrument software to misattribute CDOM fluorescence to Chl-a, or vice versa. We are aware of few studies that have purposefully examined the influence of NPQ on phytoplankton fluorescence excitation spectra or FP-based estimates of Chl-a, though many have alluded to or incidentally encountered such effects (e.g., [6, 35-38]). Unfortunately, many studies, including all of the AOA studies, have used captive samples under dim light or darkness, so they provide little evidence on the effects of NPQ when FP is used, as mainly intended, *in situ.*

The general objective of this work was to examine the effect of irradiance on the accuracy of FP fluorometry. In pursuit of this objective, we conducted laboratory experiments to characterize the potential variation in photophysiological response among phytoplankton of different pigment groups. We conducted field experiments to learn if such patterns exist for natural communities of different lakes and sites that are acclimated to different natural irradiance regimes and with varying recent light histories, and to assess irradiance effects on the accuracy of CDOM estimation. Specifically, we sought to: (1) characterize variation in the irradiance-dependency of fluorescence properties (NPQ and fluorescence excitation spectra) among cultures of different pigment groups using acute-exposure laboratory experiments, (2) assess the influence of solar radiation on the accuracy of FP estimates of Chl-a, phytoplankton taxonomy, and CDOM obtained from lakes and sites varying in prior irradiance conditions, and (3) to determine whether relatively brief exposure of natural waters to low light or darkness could reverse photophysiological adjustments made by phytoplankton *in situ*, and in so doing improve the accuracy of FP fluorometry.

METHODS

Three experimental approaches were used. First, laboratory experiments subjected monospecific cultures and binary mixtures of cultures to short-term (minutes) exposures to increasing irradiance in order to determine irradiance-response relationships reflective of NPQ induction. Second, acute-exposure experiments were performed with natural communities from five lakes (Fig. 1), to determine responses to short (15 min) exposure to high irradiance as well as responses to a subsequent period of low irradiance. Third, relaxation experiments were performed on near-surface samples from a sixth lake (Fig. 1) by applying a dark acclimation treatment to reveal effects of NPQ relaxation. Spectral fluorescence data were collected with one of two FP instruments (serial numbers: 1108, 1110). The same FP unit was used for all dates/sites within each set of experiments for consistency (SN 1108 was used in the field; SN 1110 was used in the laboratory). The terms FP-Chl-a and YS are used throughout this paper to refer to FP-based estimates of Chl-a and FP-based estimates of CDOM, respectively.

Acute Exposure-Response Experiments on Laboratory Cultures

Phytoplankton Cultures

The taxa grown in culture belong to cosmopolitan freshwater phytoplankton genera of the Laurentian Great Lakes area and represent the 4 major spectral groups distinguished by the FP (Table 1). Cells were grown in either BG-11 (*D. lemmermannii*) or WC media (all other taxa) in Erlenmeyer flasks under a 16:8 light:dark (L:D) cycle at 18°C with regular transfers to fresh media to maintain cells in exponential growth phase. The growth irradiance was approximately 40 μmol photons$\cdot m^{-2} \cdot s^{-1}$ of white light; PAR was measured with a LI-COR flat quantum sensor placed inside a Pyrex culture flask with the stopper in place. To obtain adequate sample volume

for use in the exposure-response experiments, large volumes of media were inoculated several days in advance of the experiments. For single-taxon experiments, ~2300 mL of media was inoculated in a 4000-mL flask. For mixed-taxa experiments, two 2000-mL flasks, each containing ~1300 mL of media, were inoculated; after several days of growth, 1000 mL of each culture were combined in a 3-L Pyrex beaker immediately prior to the experiment to provide the required binary taxonomic mixture.

Exposure-Response Experiments on Laboratory Cultures

Exposure-response experiments were performed 6-7 hours after the start of the light cycle. Phytoplankton cultures were exposed to a range of photon flux densities (PFDs) spanning approximately 200 to 2000 µmol photons·m^{-2}·s^{-1}. The upper end of this range is approximately equal to the surface incident PFD at solar noon in mid-summer in the study region, at which time and place the lower end of the range (200 µmol photons·m^{-2}·s^{-1}) would be encountered by *in situ* phytoplankton in an oligotrophic or meso-eutrophic lake at a depth of between approximately 2 to 13 m (depending on Chl-a and CDOM concentrations; e.g., see Table 1 in [39]). PAR was measured with a LI-COR spherical quantum sensor at two heights within the beaker, and the exact mean PAR calculated for each exposure level of each experiment based on the average vertical attenuation coefficient from all PAR levels. Illumination was produced by 2 halogen lamps positioned below the sample. The beaker was placed within a 190 × 100 mm (diameter × height) Pyrex crystallizing dish containing an ice-bath for temperature control, which maintained sample temperature within 1.5°C of initial temperature (20.8−23.4°C) throughout all exposures. Each of the series of successively-brighter exposures was 10 min in duration; the sample was stirred with a glass rod at 0, 2.5, 5, 7.5, and 10 min.

PAM Fluorometry

Prior to the start of each experiment, and after each 10-min experimental PAR exposure, a 3-mL aliquot of phytoplankton culture was transferred by pipette from the beaker to the quartz cuvette of a Water-PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany). The minimum PSII fluorescence yield in the light-acclimated state (F_O') was measured by the non-actinic (2 μmol photons m^2 s^{-1}) measuring LED of the emitter-detector unit of the PAM fluorometer. A saturation pulse (3650 μmol photons·m^{-2}·s^{-1} red light for 0.8 s) was then applied to saturate PSII electron transport, after which the maximum PSII fluorescence yield was measured (F_M in the absence of NPQ; F_M'' when measured in darkness following a period of illumination [40]). NPQ was calculated as

$$\frac{F_M - F_M''}{F_M''}$$

The initial PSII quantum yield (prior to acute PAR exposure) was calculated as

$$\frac{F_M - F_O'}{F_M}$$

The PSII quantum yield following each acute PAR exposure was calculated as

$$\frac{F_M'' - F_O'}{F_M''}$$

For more detailed information on variable fluorescence methods and technology, the reader is encouraged to consult one of the many comprehensive reviews on the subject (e.g., [18, 40, 41]).

FP Fluorometry

FP measurements were made in the aforementioned 3-L Pyrex beaker while both the FP and beaker were covered in black plastic to avoid interference from overhead fluorescent lighting.

Ten consecutive measurements were made over a 50-s period; a relatively low measurement frequency of 1 measurement every 5 s was used to avoid fluorescence induction effects from the FP's LEDs. These data were averaged and the averages used for further analysis. To obtain reference spectra for each species, FP measurements were made prior to any experimental exposure (i.e., immediately after the culture was removed from the incubator) and the fluorescence data normalized to the respective Chl-a concentration (see following section). YS was not estimated for laboratory experiments; FP measurements were corrected for dissolved fluorescence by subtracting the fluorescence signal of filtered water (see following section).

Chlorophyll-a Determination

Cultures were filtered through 47-mm glass fibre filters (Whatman GF/F; pore size = 0.7 μm) at low vacuum pressure (<0.5 atm) for determination of Chl-a concentration. The filters were immediately frozen at -20°C in glass scintillation vials and kept frozen until immediately prior to analysis. Pigment was passively extracted overnight (18-24 hours) in 90% acetone in a dark freezer (-20°C) and Chl-a determined fluorometrically (Turner Designs, 10-AU, Sunnyvale, CA, USA) with acidification to correct for phaeophytin interference [42].

Acute Solar Exposure ("Dock Box") Experiments on Lake Phytoplankton Communities

Samples were collected from the nearshore waters (<2 m depth) of five Ontario lakes (Couchiching, Fawn, Kahshe, Simcoe, St. John; Fig. 1) between 09:25 and 09:40 during late August to September 2014. A clean 19-L high-density polyethylene (HDPE) bucket was used to collect volumes of surface water that were poured through a 200-μm Nitex mesh into two 20-L plastic carboys that were stored in a cooler. A 15-L volume of sample was poured into a 5-sided, 19-L glass box and exposed to a low level of PAR provided by fluorescent light for

approximately 2 h; mean PAR (from the top to the bottom of the glass box) was ~44 µmol photons·m^{-2}·s^{-1} (determined using a flat LI-COR quantum sensor). To induce NPQ, the box containing the sample was thereafter placed at the end of an exposed (unshaded) dock at approximately (within 30 min of) solar noon for 15 min. The water was stirred every 5 min with a glass rod. Incident solar PAR was measured continuously with a cosine radiometer designed for field use (BIC-2104, Biospherical Instruments Inc., San Diego, CA, USA) positioned next to the sample. FP measurements of Chl-a and YS were made immediately prior to ('Initial') and after the solar exposures ('Exposed'), and after 45 min exposure to low PAR ('Low PAR'; ~44 µmol photons·m^{-2}·s^{-1}). FP-Chl-a was calculated using the factory-default reference spectra. Ten consecutive measurements were made at a 5-s interval. These data were averaged and the averages used for further analysis. The minimum and maximum PSII fluorescence yields were determined using a Water-PAM before and after the exposures, and at 15-min intervals following exposure while NPQ was allowed to relax under low PAR.

Samples were preserved for Chl-a, DOC, and phytoplankton taxonomy using standard methods. Samples for phytoplankton taxonomy were fixed with Lugol's iodine (3% final concentration) and stored in a dark refrigerator (4°C) until microscopic examination by a professional taxonomist. Subsamples were settled for a minimum of 8 h in a 5-mL Utermöhl counting chamber. Cells were identified using an inverted microscope (Nikon Model MS) at 100–600x magnification. Biomass estimates were based on cell measurements, following the procedures listed in the Ontario Ministry of the Environment Phytoplankton Methods Manual [43]. To determine Chl-a and DOC concentrations, water was filtered through ashed (5 h at 500°C) 47-mm Advantec GF75 filters (AMD Manufacturing Inc.; pore size = 0.3 µm) under low vacuum pressure (<0.5 atm). A portion of the filtrate was stored refrigerated in acid-washed 30-mL

amber vials until analysis for DOC concentration by catalytic oxidation at 680°C with a Shimadzu TOC-L analyzer (Shimadzu Corporation). A 3-mL aliquot of the remaining filtrate was used to correct the PAM data for dissolved fluorescence (i.e., the fluorescence yield of the filtrate was subtracted from the total fluorescence yields obtained from whole water samples to provide the Chl-a fluorescence yields). Chl-a concentration was determined as described in the preceding section.

Relaxation Experiments on Natural Communities from Coastal Georgian Bay

Data were collected from 19 sites along the southeastern shore of Georgian Bay, Lake Huron during 15 to 24 September 2014. All FP-Chl-a estimates were made using the instrument-default reference spectra. At each site, a vertical profile (surface to bottom) was first collected using the FP. Next a clean 19-L HDPE bucket was used to collect surface water which was immediately poured into a 4-L Nalgene bottle (with the upper portion cut off to form a cup) in which the FP was placed and covered in thick black plastic prior to taking measurements. A high logging frequency (s^{-1}) was used to ensure adequate data capture during profiling; the risk of fluorescence induction by the FP's LEDs was considered low given the recent light history (and presumed photoacclimation status) of the natural phytoplankton being analyzed. The sample was then poured into an opaque 2-L Nalgene bottle and stored at ambient temperature for >35 min to allow NPQ to relax, after which time a second set of FP measurements were made. Water was filtered through ashed (4 h at 475°C) 47-mm Advantec GC50 filters (AMD Manufacturing Inc.; pore size = 0.5 µm) at low vacuum pressure (<0.5 atm) for determination of Chl-a and DOC concentrations. The filters were frozen immediately on dry ice and, after return to the laboratory, stored in the dark at -20°C until analysis for Chl-a, as described above. Filtrate was stored in amber glass bottles in a cooler on the ship and thereafter transferred to a dark refrigerator until

analysis for DOC, as described above. Measurements of down-welling PAR were made from the water surface to between 1.4 and 18.2 m (as determined by site depth and/or water clarity) using the BIC-2104 radiometer described previously. Samples from 14 of the sites were analyzed for phytoplankton taxonomy by microscopy (this was not possible for all sites due to funding constraints). Integrated water samples were collected using a weighted glass bottle that was lowered to twice the Secchi depth, uncovered (by pulling a line fixed to the stopper), and retrieved at a constant rate, allowing it to fill proportionately with water from all depths without completely filling. Sample preservation and microscopy were performed as described in the previous section.

Data Analysis

The software R was used to produce figures and for statistical analysis [44].

Linear Unmixing

Estimates of group-specific Chl-a and of YS were obtained from FP fluorescence excitation spectra and reference spectra using non-negative least squares estimation, as described in Harrison et al. [45]; this 'linear unmixing' approach is the same method used by the FP software [4] and is described in detail elsewhere [5]. Species-specific reference spectra (obtained from the cultures described above) were applied to fluorescence data from the laboratory experiments. For experiments including neither of the two taxa from the brown spectral group, an average brown spectrum was used (i.e., the spectra obtained from *F. crotonensis* and *S. petersenii* were summed and divided by 2). The instrument-default reference spectra (including a YS spectrum) were applied to data obtained from natural systems. Statistical methods exist for obtaining more accurate, system-specific reference spectra for certain environments (e.g., [45]). We chose not to

(attempt to) derive custom reference spectra for this study so that the error in FP data associated with light history could be interpreted relative to the baseline inaccuracy of the FP as it is commonly used in the field (i.e., with the factory-default reference spectra). The FP used in the field studies had recently (April 2014) been shipped to bbe Moldaenke GmbH for assessment and met their quality control standards.

Taxonomic Classification Error

For the field studies, classification error of the FP was calculated based on microscopy-based estimates of phytoplankton biomass as the mean squared prediction error for each site

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(\hat{Y}_i - Y_i\right)^2$$

where n is the number of groups (i.e., 4), $\hat{Y}_i$ is the predicted (FP) proportion of group i and Y_i is the observed (microscopy-based) proportion of group i.

Comparison of Means

FP-Chl-a and YS data collected after dock box exposures and after 45 min of low PAR were compared to pre-exposure data using Wilcoxon rank sum tests (df = 9). This nonparametric test was used in place of t-tests because not all of the data conformed to a normal distribution (as determined using the Shapiro-Wilk test [46]). The MSE of taxonomic classification for Georgian Bay surface samples before and after dark acclimation was compared using a paired t-test (df = 13).

Regression Analyses

Simple linear regression (SLR) was used to assess the accuracy of the FP in predicting extracted Chl-a, DOC, and taxonomic composition of the phytoplankton community (Georgian Bay data)

and to assess whether PAR-dependent changes in FP-Chl-a during laboratory experiments were better explained by F_O', F_M'', NPQ, or PSII quantum yield. The PFD of PAR immediately below the surface (E0) and the vertical attenuation coefficient for PAR (KD_{PAR}) at sites on Georgian Bay were calculated based on the intercepts and slopes, respectively, from SLR analyses of the natural logarithm of down-welling PAR as a function of water column depth. Multiple linear regression was used to quantify the variation in FP data explained by E0 and KD_{PAR}: the changes in FP-Chl-a and YS due to dark acclimation (i.e., the light-acclimated to dark-acclimated ratio for each parameter) and the MSE of taxonomic assignment for light- and dark-acclimated samples were modeled as a function of E0 and KD_{PAR}. Data were natural log-transformed to normalize residuals, if necessary [47]. The normality of the residual error from regressions was assessed using the Shapiro-Wilk test.

RESULTS

Acute Exposure-Response Experiments on Laboratory Cultures

Fluorescence vs. irradiance responses of cultures varied considerably among taxa (Fig. 2a). The synurophyte *S. petersenii* (brown group) exhibited the most NPQ and greatest declines in F_O', PSII quantum yield, and FP-Chl-a with increasing irradiance (Fig. 2e). In contrast, *Cryptomonas sp.* (red group) showed relatively little NPQ but large increases in F_O' and FP-Chl-a with increasing irradiance (Fig. 2b). Simple linear regression analysis of the pooled FP and PAM data from all PAR exposures of the 5 monoculture experiments (i.e., data in Fig. 2; df=74) revealed that the PAR-dependent change in F_O' (as measured by Water-PAM) was a better predictor of the change in FP-Chl-a (R^2=0.47; p<0.001; slope=0.87; intercept=0.12) than the change in F_M'' (R^2=0.15; p<0.001; slope=0.43; intercept=0.69), NPQ (R^2=0.24; p<0.001; slope=-0.19; intercept=1.11), or PSII quantum yield (R^2=0.21; p<0.001; slope=0.82; intercept=0.44). PAR-

induced changes in the fluorescence excitation spectra (Fig. 3) led to relatively minor error in taxonomic classification of monocultures (average = 3.2%; Table 3). The error (i.e. the percentage of Chl-a assigned incorrectly) varied with taxon and PAR exposure level, but was not always higher for a given taxon at higher PAR (e.g., highest error of 11.6% was for the chlorophyte *P. simplex* at only 187 μmol photons·m^{-2}·s^{-1}).

Binary taxonomic mixtures exhibited taxon-specific PAR-dependent changes in FP-Chl-a (Fig. 4) that were generally consistent with those observed during monoculture experiments (Fig. 2a). Mixtures including *S. petersenii* underwent large irradiance-dependent changes in FP-Chl-a: the fraction assigned to *S. petersenii* increased with increasing irradiance, while the FP-Chl-a of the other taxon of the binary mixture (*P. simplex*, *D. lemmermannii*) was relatively consistent in each experiment. FP attributed the majority of the Chl-a to the correct pigment groups, but a small proportion was assigned erroneously (i.e., to a group not present) in all four of the binary mixture experiments.

Acute Solar Exposure ("Dock Box") Experiments on Lake Phytoplankton Communities

The lakes varied in Chl-a (1.0 to 34 μg·L^{-1}) and DOC (3.8 to 10 μg·L^{-}) concentrations (Table 2). Microscopy-based estimates of biomass distribution among pigment groups typically differed from the FP estimates of Chl-a distribution as measured before the acute (15-min) experimental solar irradiance exposures ('FP initial'; Fig. 5). The accuracy of taxonomic classification by FP after the solar exposures was unrelated to the irradiance history of the community (Fig. 6). In some lakes (Fawn and Kahshe), the disparity between taxonomic composition based on biomass and on FP prior to solar exposure was clearly much larger than the change in FP-based taxonomy induced by the exposure (Figs. 5b, 5c). By contrast, microscopy- and FP-based estimates of taxonomy were very similar in Lake Couchiching, but the taxonomic distribution of FP-Chl-a

changed considerably in response to irradiance (Fig. 5a). The remaining lakes (St. John, Simcoe) showed considerable initial disparity of biomass vs FP-Chl-a, as well as substantial changes in FP-Chl-a (Figs. 5d, 5e). Irradiance-induced changes in total FP-Chl-a varied among lakes (Fig. 5f). Interestingly, high irradiance increased red FP-Chl-a in Lake Couchiching and decreased the brown FP-Chl-a (Fig. 5a), but the opposite pattern was observed for Lake Simcoe (Fig. 5d). In both Couchiching and Simcoe, the 45-min-low-irradiance treatment following the 15-min-high-irradiance exposure tended to reverse the high irradiance effects on FP-Chl-a distribution. The high-irradiance exposure led to a statistically-significant (p<0.05) increase in FP-Chl-a for Lakes Simcoe and Couchiching, and to significant decreases in YS for all 5 lakes. After 45 min of low PAR, FP-Chl-a was significantly (p<0.05) higher than prior to exposure for the 2 lakes which had the highest-PFD solar exposures, Couchiching and St. John (Figs. 5a, 5d). After 45 min of low PAR, YS remained significantly (p<0.05) lower than it was prior to solar exposure for all lakes except Kahshe (p=0.09).

Relaxation Experiments on Phytoplankton Communities from Coastal Georgian Bay

FP-Chl-a was a significant (p<0.001) predictor of extracted Chl-a (Table 2) before and after dark acclimation (Fig. 7a). The average change in FP-Chl-a due to dark acclimation was an increase of 44% (range of -13% to 145%), whereas dark acclimation generally had little effect on YS (Fig. 7b). FP-Chl-a measurements made immediately after sample collection showed better agreement with extracted Chl-a (R^2=0.93; slope=0.88; intercept=-0.31) than those made after dark acclimation (R^2=0.79; slope=0.80; intercept=-0.77). YS was a significant (p<0.001) predictor of DOC concentration both before (R^2=0.87; slope=2.180; intercept=1.49) and after dark acclimation (R^2=0.88; slope=2.176; intercept=1.49), with regressions producing visually-indistinguishable trend lines (Fig. 7b). Variation in the ratio of ambient-light-acclimated to dark-

acclimated FP-Chl-a of the surface water samples could be significantly (p<0.001) explained by variation in E0 and KD_{PAR} among sample sites, as determined by multiple linear regression analysis, whereas no such relationship (p=0.8) was observed for YS (Table 4).

Phytoplankton biomass and taxonomy based on microscopic examination of integrated samples (surface to twice Secchi depth) were compared to averaged FP profile data from the same depths. FP-Chl-a was a significant predictor of total phytoplankton biomass as estimated by microscopy (R^2=0.58; p<0.01; df=12) when both variables were log-transformed to normalize the residuals of the regression. The strength of this correlation was slightly greater than that between acetone-extracted Chl-a and total phytoplankton biomass when both variables were log-transformed (R^2=0.52; p<0.01; df=12). FP-based community composition (i.e., proportion FP-Chl-a per group) was a significant predictor of microscopy-based estimates (i.e., relative biomass per group) for brown FP-Chl-a vs. heterokont/dinoflagellate biomass (R^2=0.29; p<0.05), but not green FP-Chl-a vs. chlorophyte/euglenoid biomass (R^2=0.06, p=0.41), red FP-Chl-a vs. cryptophyte biomass (R^2=0.03; p=0.59), or blue FP-Chl-a vs. cyanobacteria biomass (R^2=0.06; p=0.39). Interestingly, the red fraction of the total FP-Chl-a was a significant predictor of the relative cyanobacteria biomass (R^2=0.34, p<0.05), and likewise, the blue fraction of the total FP-Chl-awas a significant predictor of the fraction of cryptophyte biomass (R^2=0.62, p<0.001).

FP estimates of community composition of surface samples before ('FP – Light') and after ('FP – Dark') dark acclimation both showed much lower representation by blues and greens than would be expected based on microscopic examination of integrated samples (Fig. 8). Dark acclimation of the surface samples generally increased the proportion of browns relative to reds (Fig. 8). The MSE of taxonomic classification showed a significant positive correlation with surface incident PAR for samples analyzed immediately after collection; this relationship was no

longer significant after dark acclimation (Fig. 6, Table 4). The average taxonomic classification error (MSE) for dark acclimated samples (0.073) was significantly lower than for light-acclimated samples (0.090) based on a paired t test ($p<0.05$).

DISCUSSION

We performed three sets of experiments to investigate how recent light history affects the fluorescence emission spectra of phytoplankton and what the implications could be for use of the FP and related multi-wavelength fluorometers.

Considerable variation in the irradiance-dependence of fluorescence emission (quantified by FP and PAM fluorometry) was observed for the 5 phytoplankton taxa exposed to a range of PFDs of PAR under laboratory conditions. All cultures were growing in exponential phase with PSII quantum yield values near the expected/empirical maximum for healthy eukaryotic [48] and cyanobacterial [49] cells, indicating that nutrient limitation or other stressors were not a factor prior to the experimental light exposures. While all taxa exhibited a decrease in photochemical efficiency and the induction of at least some NPQ at high PFDs, the effect on FP-Chl-a was variable not only in magnitude, but in direction: FP-Chl-a of *Cryptomonas* sp. and the chlorophyte *P. simplex* increased with increasing PFDs of PAR, whereas FP-Chl-a of the synurophyte and diatom decreased substantially, and there was little effect on the cyanobacterium *D. lemmermannii*.

We are aware of only one other study that has assessed the effects of acute light stress on fluorescence excitation spectra and the consequences for group-specific estimates of phytoplankton Chl-a. MacIntyre et al. [4] induced NPQ in cultures of a chlorophyte (*Dunaliella tertiolecta*), cryptophyte (*Rhodomonas lens*), cyanobacterium (*Synechococcus bacillaris*), and 3

algae from the brown pigment group (*Thalassiosira pseudonana*, *Amphidinium carteraei*, and *Emiliania huxleyi*) with 40-50 min exposures of 800 μmol photons·m^{-2}·s^{-1} (10× the growth irradiance) and monitored changes in readings obtained by the AOA. These authors observed no appreciable (>1%) change in taxonomic assignment by the AOA due to high PAR exposure. This finding is generally consistent with the relatively low error in taxonomy estimates we observed during monoculture experiments (1-6%; see Table 3). In contrast to our results, these authors noted a quenching of the estimated Chl-a for taxa from all pigment groups, whereas we observed highly variable responses: for example, there was strong stimulation of FP-Chl-a for *Cryptomonas sp*. The level of NPQ induced, and associated reduction in FP-Chl-a observed by MacIntyre et al. [4] for the diatom *T. pseudonana* (reduction in estimated Chl-a of ~20%; NPQ of ~0.6; data from other taxa were not reported) were of comparable magnitude to what we observed for the diatom *F. crotonensis* when it was exposed to approximately 10× the growth irradiance (see Fig. 2a, e). In another study employing lab cultures, Escoffier et al. [7] compared dark- and light-acclimated freshwater phytoplankton cultures (using different species from two of the same genera that we assessed: *Cryptomonas* and *Pediastrum)* and observed a reduction in the FP-Chl-a of all taxa, ranging from 2 to 29%, for the 5 strains assessed. Though these responses were attributed to NPQ, the PFDs of the light treatments used in this study were relatively low (equal only to the growth irradiance of 20-30 μmol photons·m^{-2}·s^{-1}); thus their experimental design and results are not directly comparable to the present study.

The lab culture experiments showed that FP-Chl-a estimates were changed in taxon-specific ways as PAR exposure increased, and the changes were better-predicted by the minimum (F_O') than by the maximum (F_M'') light-acclimated PSII fluorescence yield (measured in the dark). It would therefore appear that the level of fluorescence measured by the FP is closer to

the minimum PSII fluorescence than the maximum, in contrast to what has been stated elsewhere (cf. [7]). It required at least a ten-fold increase of PAR above growth irradiance to alter F_O' enough to change FP-Chl-a estimates by 20% or more. While such dynamics of light exposure may appear extreme, vertical mixing and cloud passage can also produce large and rapid variations in sunlight exposure in natural communities (e.g. [50, 51]). The experimental treatments used here also helped to reveal the variations of response that can exist among phytoplankton of different genera and pigment groups. The similar responses of the diatom *F. crotonensis* and the chrysophyte *S. petersenii* may reflect a shared disposition of algae of the brown group to exhibit relatively high NPQ and thus quenching of PSII fluorescence [30, 52]. The increase in brown FP-Chl-a due to dark acclimation, as observed in Georgian Bay, would also be consistent with relaxation from a high level of NPQ compared to other groups. However, the results of the dock box experiments suggest that the responses of FP-Chl-a estimates for pigment groups in natural communities can vary. For instance, solar exposure shifted the apparent community composition of Lake Couchiching away from browns and toward reds, consistent with patterns observed in lab cultures, but the opposite pattern was observed in Lake Simcoe. In L. Couchiching the dominant cryptophyte (94% of cryptophyte biovolume as determined by microscopic analysis) was *Cryptomonas,* which in culture showed increased F_O' and FP-Chl-a under light stress. In L. Simcoe, the dominant (88%) cryptophyte was instead *Rhodomonas,* a genus that can show high NPQ and sensitivity to photoinhibition [53]. The effect of sunlight stress on FP-Chl-a for different co-occurring pigment groups in natural communities may depend both on group-specific tendencies and on taxon-dependent and/or habitat-specific variations, as suggested by some previous work (e.g., [54, 55]).

We observed statistically-significant evidence for effects of irradiance on FP estimates of both total Chl-a and taxonomic composition. On average, the FP-Chl-a of Georgian Bay surface samples, which were largely dominated by browns, increased after >35 min of dark acclimation (Fig. 7), and the magnitude of this change was correlated with light history among sites (Table 4). By comparison, Serra et al. [13] showed that FP-Chl-a in lake communities dominated by browns (diatoms in particular) progressively underestimated the *in vitro* Chl-a values as irradiance increased from low to moderate values. At high irradiance (i.e., at depths less than 2.5 m from the surface), FP-Chl-a had a more variable relationship with *in vitro* Chl-a, but on average continued to give under-estimates [13]. Those results suggested that effects of high irradiance can introduce variability in expression of NPQ and resultant FP-Chl-a. The correlation between FP-Chl-a and extracted Chl-a was indeed weaker for our non-dark acclimated Georgian Bay samples than for the dark-acclimated samples, and not only at high irradiance, but across a range of prior PAR exposures. Leboulanger et al. [38] showed strong (40%) reductions of FP-Chl-a estimates in a cyanobacteria-dominated community under moderate winter levels of near-surface irradiance. Even a moderate irradiance stress may therefore introduce variability into FP-Chl-a estimates for some communities. We note that FP-Chl-a of most Georgian Bay samples, both light- and dark-acclimated, was somewhat higher than extracted Chl-a; this difference could in part be due to incomplete pigment extraction by the passive acetone extraction methodology we used [56].

The accuracy of taxonomic discrimination was negatively, linearly correlated with incident irradiance in Georgian Bay. While dark acclimation improved the taxonomic accuracy somewhat, the FP generally did a poor job of estimating phytoplankton community composition at the Georgian Bay sites, as it did in Fawn Lake and Lake Simcoe. These results are consistent

with previous studies that have observed appreciable error in taxonomic assignment using FP [7, 57] and related multi-wavelength fluorometers [4, 23]. The observation that red FP-Chl-a was a significant predictor of the relative cyanobacteria biomass and that blue FP-Chl-a was a significant predictor of the relative cryptophyte biomass in Georgian Bay could reflect the presence of phycoerythrin-rich cyanobacteria [6] and blue-green cryptomonads [58], or inaccuracy in the red spectral region of the default reference spectra we utilized.

One of the advantages commonly associated with the FP and related instruments is the ability to comprehensively characterize spatial variability (e.g. vertical profiles, nearshore-offshore gradients, etc.) in phytoplankton abundance and composition across large scales (e.g., [5, 6]). To what extent then, is the accuracy of such data likely to be undermined by the inherently-variable underwater light climate? Our results suggest that while error in taxonomic assignment tends to be greater under higher irradiance under natural conditions (see Fig. 5), the magnitude of the irradiance effect is small relative to the baseline inaccuracy of the instrument (e.g., see Fig. 8) when used with the factory-default reference spectra (as it was used for our field (but not lab) experiments), and when red FP-Chl-a is assumed to represent only cryptophytes and blue FP-Chl-a all cyanobacteria (i.e., as reported by the FP software). Based on our results, we can only tentatively recommend dark acclimation of natural samples (or exposure to dim light [41]), if practical, as it may increase the overall accuracy of taxonomic estimates, though not for all pigment groups. It is certainly clear from our results that the instrument with default spectra gave misleading estimates of community composition, and the use of representative spectra is recommended when possible [5, 45, 59], though this may be logistically challenging for those engaged in routine water quality monitoring.

Conclusions

Pronounced taxonomic variability in NPQ and in irradiance-induced changes to FP estimates were observed when working with laboratory cultures. Under natural conditions the FP effectively quantified patterns in total Chl-a and DOC under a wide range of irradiance conditions. Estimates of phytoplankton taxonomy were generally of low accuracy and the accuracy was negatively correlated with incident irradiance in Georgian Bay. We conclude that for those using the FP to measure total and group-specific Chl-a in the field, applicability of the reference spectra to the ecosystem under study is likely to be a more important consideration than is variability in the light regime and its potential effects on phytoplankton fluorescence, though such effects can contribute to systematic inaccuracy in FP data and should be recognized. Our study lakes were predominantly oligotrophic; further studies in meso-eutrophic systems (e.g., [60]) are needed.

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Table 1. Phytoplankton cultures used in acute irradiance exposure laboratory experiments.

Taxon	Phytoplankton Group	Spectral Group	Culture Source	Media
Dolichospermum lemmermannii* LO08-01	Cyanobacteria	Blue	CCIW	BG-11
Pediastrum simplex Meyen CPCC 431	Chlorophyta	Green	CPCC	WC
Fragilaria crotonensis Kitton CPCC 269	Bacillariophyceae	Brown	CPCC	WC
Synura petersenii Korshikov CPCC 495	Synurophyceae	Brown	CPCC	WC
Cryptomonas sp. CPCC 336	Cryptophyta	Red	CPCC	WC

*formerly called *Anabaena*

CCIW – culture obtained from Dr. Susan Watson of the Watershed Hydrology and Ecology Research Division (WHERD) at the Canadian Center for Inland Waters, (CCIW, Burlington ON)

CPCC – cultures obtained from the Canadian Phycological Culture Center (CPCC, University of Waterloo)

Table 2. Chl-a (acetone-extracted), and DOC of the study lakes.

Lake	Date(s) (2014)	Chl-a (µg/L)	DOC (mg/L)
Couchiching	26 August	1.0	4.3
Kahshe	10 October	2.9	5.8
Fawn	27 August	2.7	10
Simcoe	9 October	2.1	3.8
St. John	28 August	34	6.1
Georgian Bay, Lake Huron	15-24 September	2.0 (0.9-6.2)	2.6 (1.6-5.0)

NB: Averages and ranges are shown for the 19 sites on Georgian Bay, Lake Huron.

Table 3. Effect of increasing PAR on inaccuracy of taxonomic assignment (% total FP Chl-a misattributed, as AVG±SD for each PAR range) during lab monoculture exposure experiments.

	Inaccuracy of taxonomic assignment (%)		
Taxon	PAR range (µmol photons m^{-2} s^{-1})		
(spectral group)	Low (187−404)	Medium (424−877)	High (905−2228)
F. crotonensis (brown)	1.2	2.0 ± 1.1	4.5 ± 2.2
S. petersenii (brown)	4.1 ± 0.9	2.3 ± 2.0	3.9 ± 0.7
P. simplex (green)	5.8 ± 5.8	2.4 ± 1.1	3.8 ± 0.2
C. sp.(red)	3.7 ± 4.7	4.2 ± 1.7	1.0 ± 0.3
D. lemmermannii (blue)	1.3	1.9 ± 2.2	3.9 ± 0.1

Note: PAR ranges correspond to values in the 0-33rd, 34th-66th, and 67th-100th percentile ranges, respectively. Values not followed by SD are single values.

Table 4. Results of multiple linear regression analyses of FP and PAR data from 19 sites on coastal Georgian Bay. The relative changes in FP-Chl-a (i.e., Chl-a-light / Chl-a-dark) and YS (YS-light / YS-dark) due to dark acclimation and the MSE of taxonomic assignment for light-acclimated samples (MSE FP-light) and dark-acclimated samples (MSE FP-dark) were modeled as a function of subsurface PAR (E0) and the vertical attenuation coefficient for PAR (KD_{PAR}).

Dependent Variable	Independent Variables						Multiple Regression	
	E0			KD_{PAR}				
	coefficient	SE	*p*	coefficient	SE	*p*	R^2_{adj}	*p*
Chl-a-light / Chl-a-dark	-0.19	0.05	**0.0014**	0.22	0.08	**0.013**	0.59	**<0.001**
YS-light / YS-dark	0.009	0.03	0.79	-0.03	0.05	0.59	0.00	0.80

MSE FP-dark	0.00005	0.00004	0.18	-0.02	0.06	0.81	0.21	0.28
MSE FP-light	0.00009	0.00004	**0.03**	0.009	0.06	0.88	0.41	0.06

Note: Variables were $\log_e$ transformed to normalize residuals for the first two regressions.

Figure 1. Study sites (blue dots) on lakes in Ontario, Canada. Fawn Lake is not visible due to its small size.

Figure 2. Change in FP-Chl-a, PSII quantum yield, NPQ, and F_O' with PAR exposure, as measured during each of the 5 monoculture experiments. (a) Points represent arithmetic means of 10 consecutive FP measurements of Chl-a; error bars represent standard deviations; both are scaled to mean values at the growth irradiance (~40 μmol photons m^{-2} s^{-1}) (b)-(f) Grey boxes, black circles, and white diamonds represent NPQ, PSII quantum yields, and F_O', respectively, from single measurements made by a Water-PAM fluorometer. The percentage shown is F_O' at the highest PAR level relative to F at the lowest PAR level.

Figure 3. Effects of PAR on FP spectra during monoculture experiments. Lighter shading of points indicates a higher PAR exposure level (legend shows PAR in μmol photons m^{-2} s^{-1}).

Figure 4. Chl-a (first column) and FP-Chl-a per phytoplankton group as a function of PAR (subsequent columns) during mixture experiments (*Cryptomonas* sp. in red, *D. lemmermanni* in blue; *S. petersenii* in brown, and *F. crotonensis* in yellow).

Figure 5. Relative composition of phytoplankton communities from 5 Ontario lakes ("Orillia Lakes") as inferred by microscopy (blue=cyanobacteria; red=cryptophytes; brown=heterokonts+dinoflagellates; green=chlorophytes+euglenoids) and FP (colors correspond to spectral groups), and the effects of 15-min solar exposures followed by 45-min low-PAR exposures on NPQ (black squares) and FP data. The numbers in parentheses following the lake names represent the average and maximum incident PAR (μmol m^{-2} s^{-1}) recorded during the 15-min exposures. The scatterplot (panel f) depicts the relationship between max. PAR and the

change in FP-Chl-a (circles) and YS (squares), relative to initial values, after solar exposure (colored symbols) and a subsequent 45-min exposure to low PAR (white symbols).

Figure 6. Accuracy of taxonomic assignment by FP as a function of phytoplankton irradiance history in the Orillia-area lakes and Georgian Bay. The x-axis represents maximum incident PAR recorded during each 15-min exposure for the Orillia Lakes dataseries; for the Georgian Bay dataseries, the x-axis represents PAR immediately below the surface of the water (E0) at the time of sample collection.

Figure 7. FP estimates of Chl-a and DOC in surface water collected from coastal Georgian Bay in September 2014. (a) Extracted Chl-a as a function of FP-Chl-a before ('light') and after ('dark') a dark acclimation period of >35 mins. The solid, dashed, and dotted lines represent the 1-to-1 line, and the trend lines from simple linear regressions (SLRs) for the light and dark dataseries, respectively. (b) DOC as a function of YS before and after dark acclimation. Note that the two trend lines in (b) overlap.

Figure 8. Georgian Bay phytoplankton community composition, as estimated by FP, before ('FP – Light') and after ('FP – Dark') dark acclimation, and by microscopy ('Biovolume'). Colors represent the different phytoplankton groups (red/cryptophytes; green/chlorophytes+euglenoids; blue/cyanobacteria; brown/heterokonts+dinoflagellates). X-axis values represent PAR (μmol photons m^{-2} s^{-1}) immediately below the water's surface at the time of sample collection.

Highlights

- FluoroProbe (FP) is widely used for the assessment of phytoplankton biomass and taxonomy
- variability in irradiance exposure potentially affects the accuracy of FP data
- irradiance effects on FP data were examined using cultures & natural phytoplankton
- we observed significant irradiance effects on FP data in the lab and in nature
- irradiance effects are unlikely to be the major cause of inaccuracy in FP data

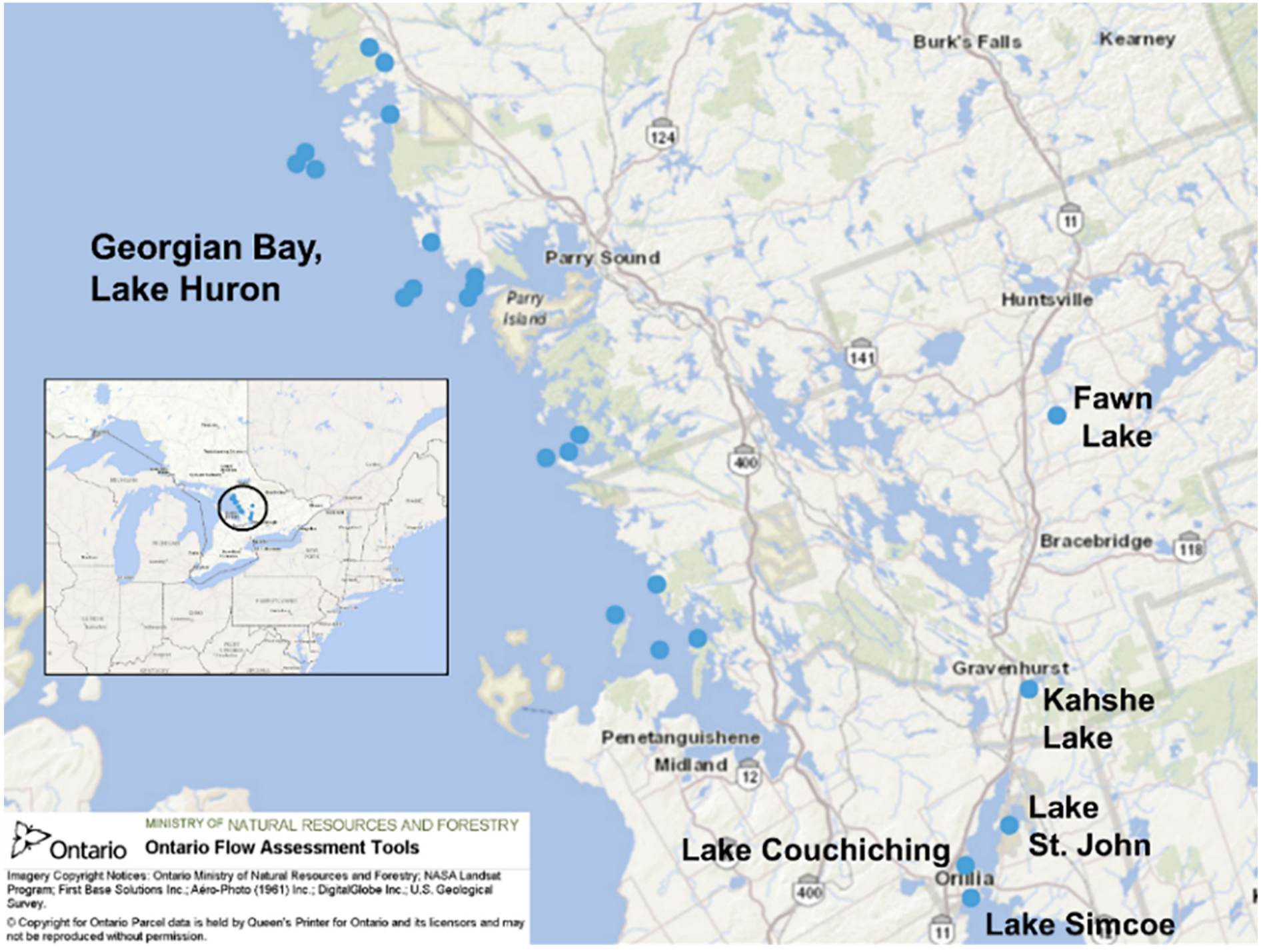
Georgian Bay,
Lake Huron
Burk's Falls
Kearney
Parry Sound
Parry Island
Huntsville
Fawn Lake
Bracebridge
Gravenhurst
Kahshe Lake
Penetanguishene
Midland
Lake St. John
Lake Couchiching
Orillia
Lake Simcoe
124
11
141
400
118
12
MINISTRY OF NATURAL RESOURCES AND FORESTRY
Ontario
Ontario Flow Assessment Tools
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Figure 1

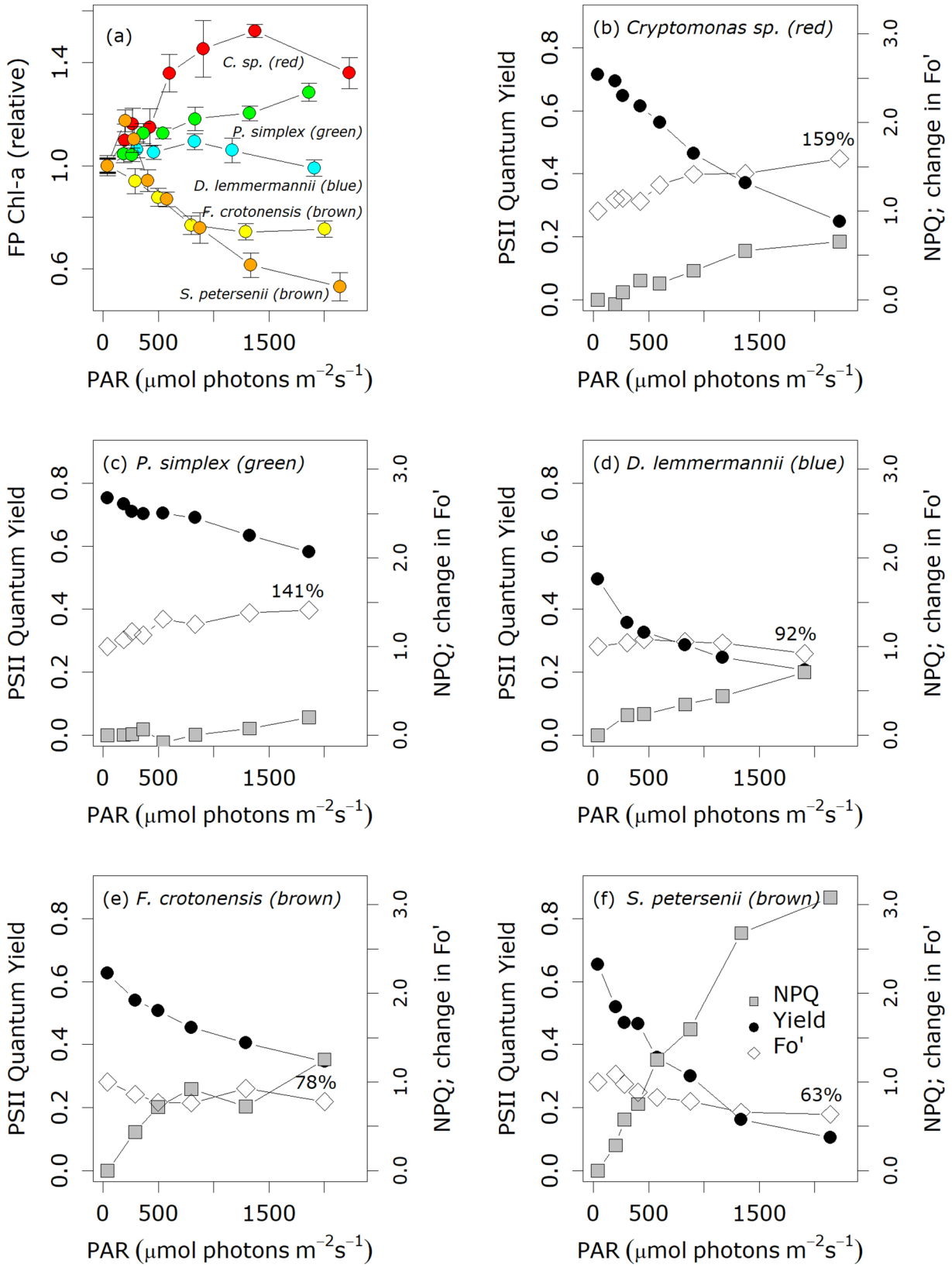
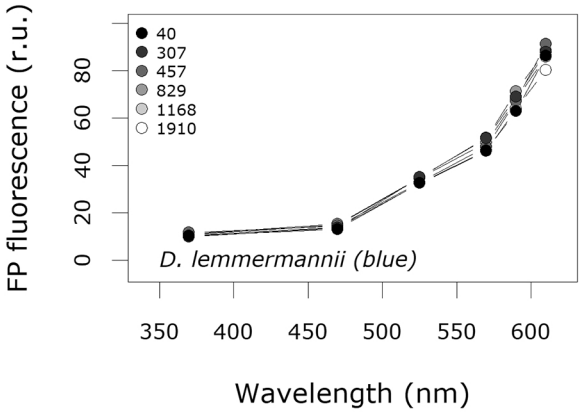
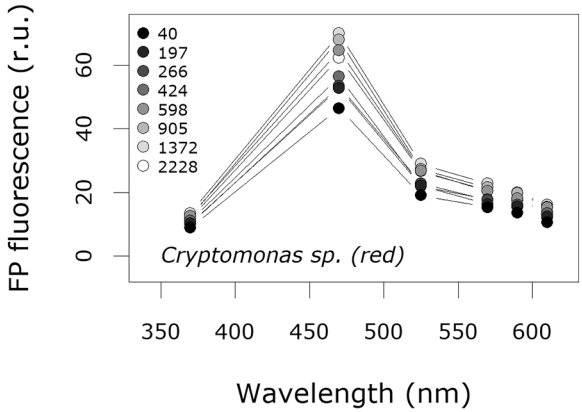
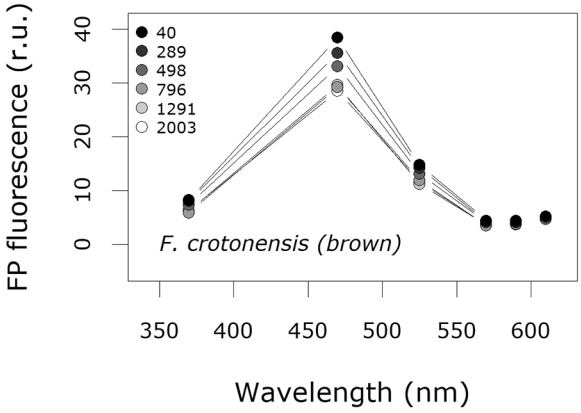
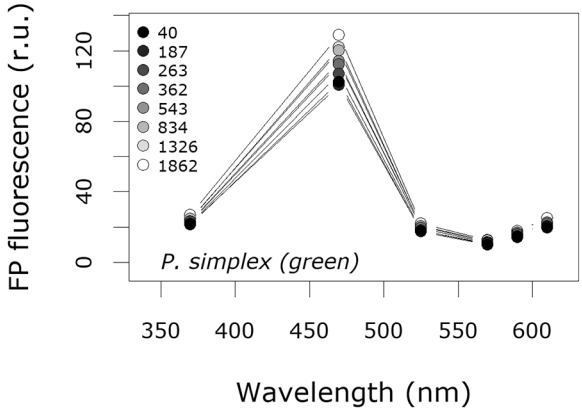

(a)
C. sp. (red)
P. simplex (green)
D. lemmermannii (blue)
F. crotonensis (brown)
S. petersenii (brown)
FP Chl-a (relative)
PAR (μmol photons m⁻²s⁻¹)
(b) Cryptomonas sp. (red)
159%
PSII Quantum Yield
NPQ; change in Fo'
(c) P. simplex (green)
141%
(d) D. lemmermannii (blue)
92%
(e) F. crotonensis (brown)
78%
(f) S. petersenii (brown)
63%
NPQ
Yield
Fo'

Figure 2


FP fluorescence (r.u.)
0
20
40
60
80
40
307
457
829
1168
1910
D. lemmermannii (blue)
350
400
450
500
550
600
Wavelength (nm)


FP fluorescence (r.u.)
0
20
40
60
40
197
266
424
598
905
1372
2228
Cryptomonas sp. (red)
350
400
450
500
550
600
Wavelength (nm)


FP fluorescence (r.u.)
0
10
20
30
40
40
289
498
796
1291
2003
F. crotonensis (brown)
350
400
450
500
550
600
Wavelength (nm)


FP fluorescence (r.u.)
0
40
80
120
40
187
263
362
543
834
1326
1862
P. simplex (green)
350
400
450
500
550
600
Wavelength (nm)

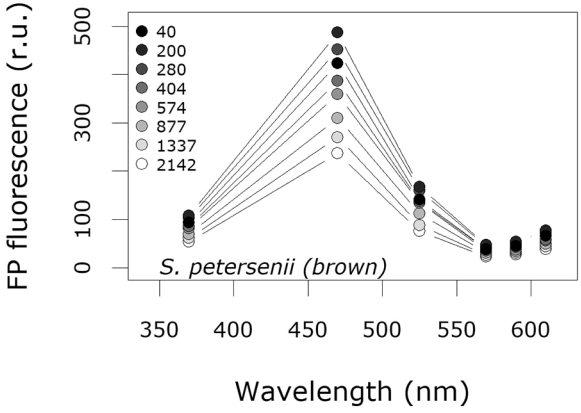

FP fluorescence (r.u.)
0
100
300
500
40
200
280
404
574
877
1337
2142
S. petersenii (brown)
350
400
450
500
550
600
Wavelength (nm)

Figure 3

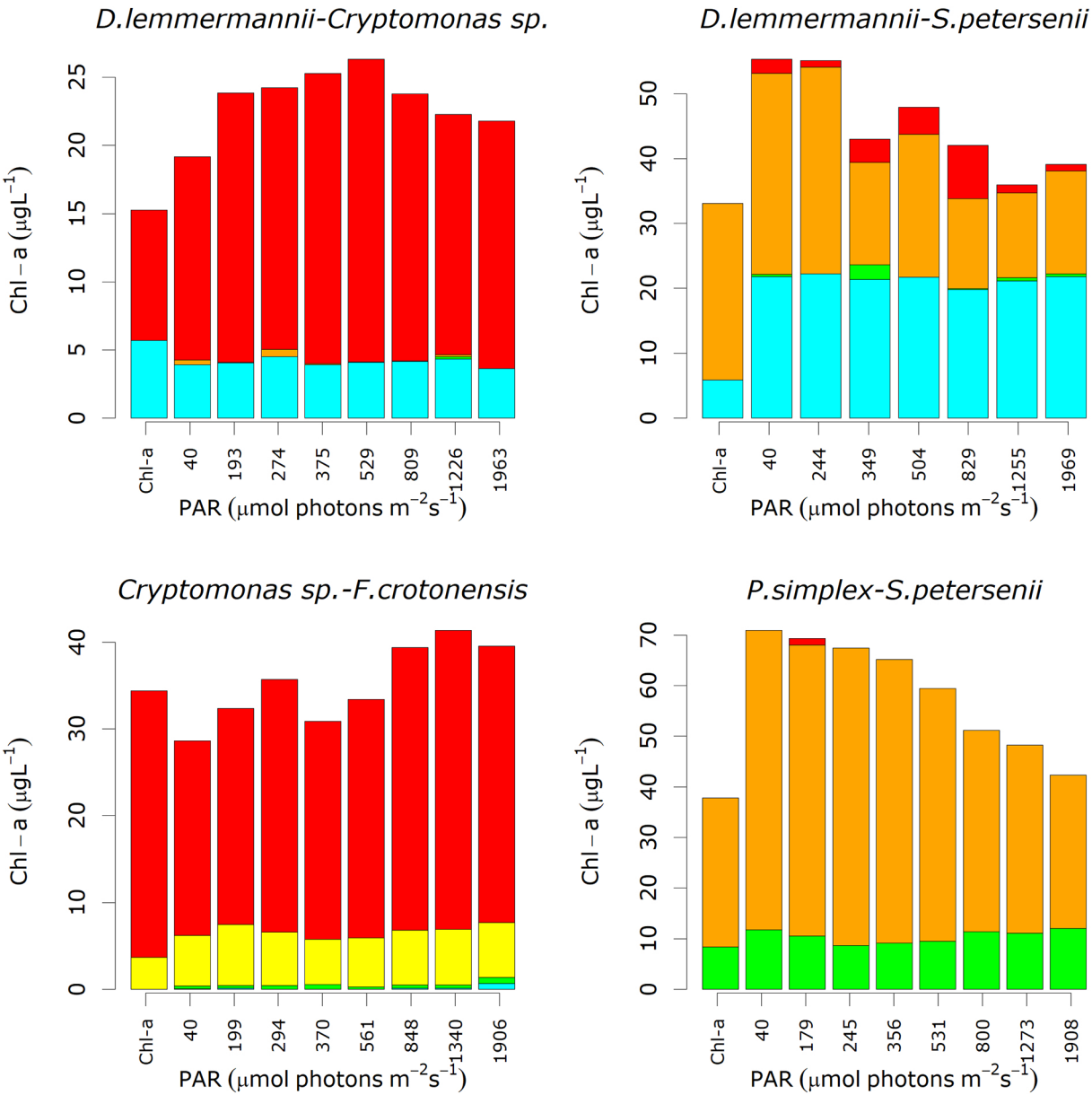

D.lemmermannii-Cryptomonas sp.
Chl – a (µgL^{-1})
0
5
10
15
20
25
Chl-a
40
193
274
375
529
809
1226
1963
PAR (µmol photons m^{-2}s^{-1})
D.lemmermannii-S.petersenii
Chl – a (µgL^{-1})
0
10
20
30
40
50
Chl-a
40
244
349
504
829
1255
1969
PAR (µmol photons m^{-2}s^{-1})
Cryptomonas sp.-F.crotonensis
Chl – a (µgL^{-1})
0
10
20
30
40
Chl-a
40
199
294
370
561
848
1340
1906
PAR (µmol photons m^{-2}s^{-1})
P.simplex-S.petersenii
Chl – a (µgL^{-1})
0
10
20
30
40
50
60
70
Chl-a
40
179
245
356
531
800
1273
1908
PAR (µmol photons m^{-2}s^{-1})

Figure 4

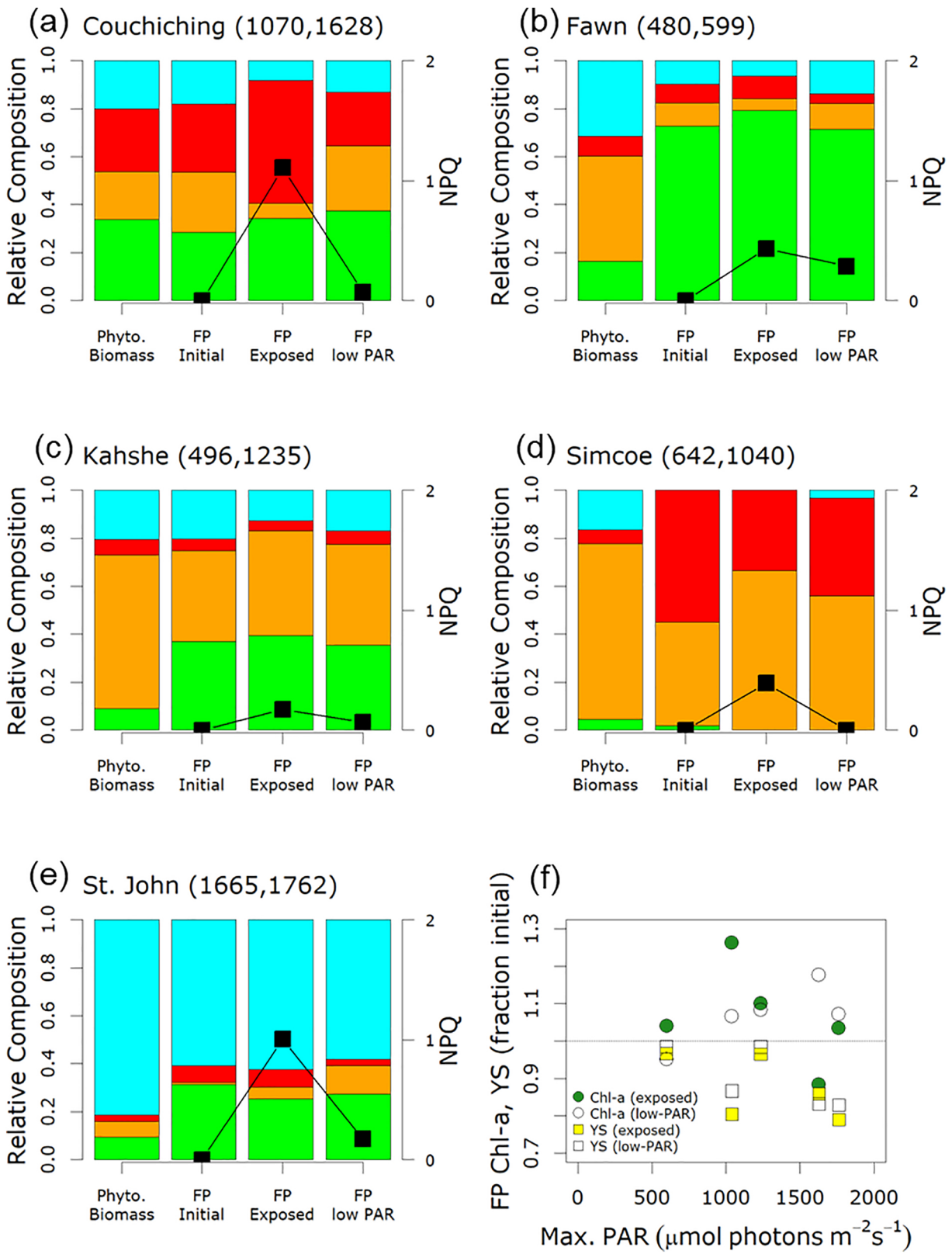

(a) Couchiching (1070,1628)
(b) Fawn (480,599)
(c) Kahshe (496,1235)
(d) Simcoe (642,1040)
(e) St. John (1665,1762)
(f)
Relative Composition
NPQ
Phyto. Biomass
FP Initial
FP Exposed
FP low PAR
FP Chl-a, YS (fraction initial)
Max. PAR (µmol photons m^{-2}s^{-1})
Chl-a (exposed)
Chl-a (low-PAR)
YS (exposed)
YS (low-PAR)

Figure 5

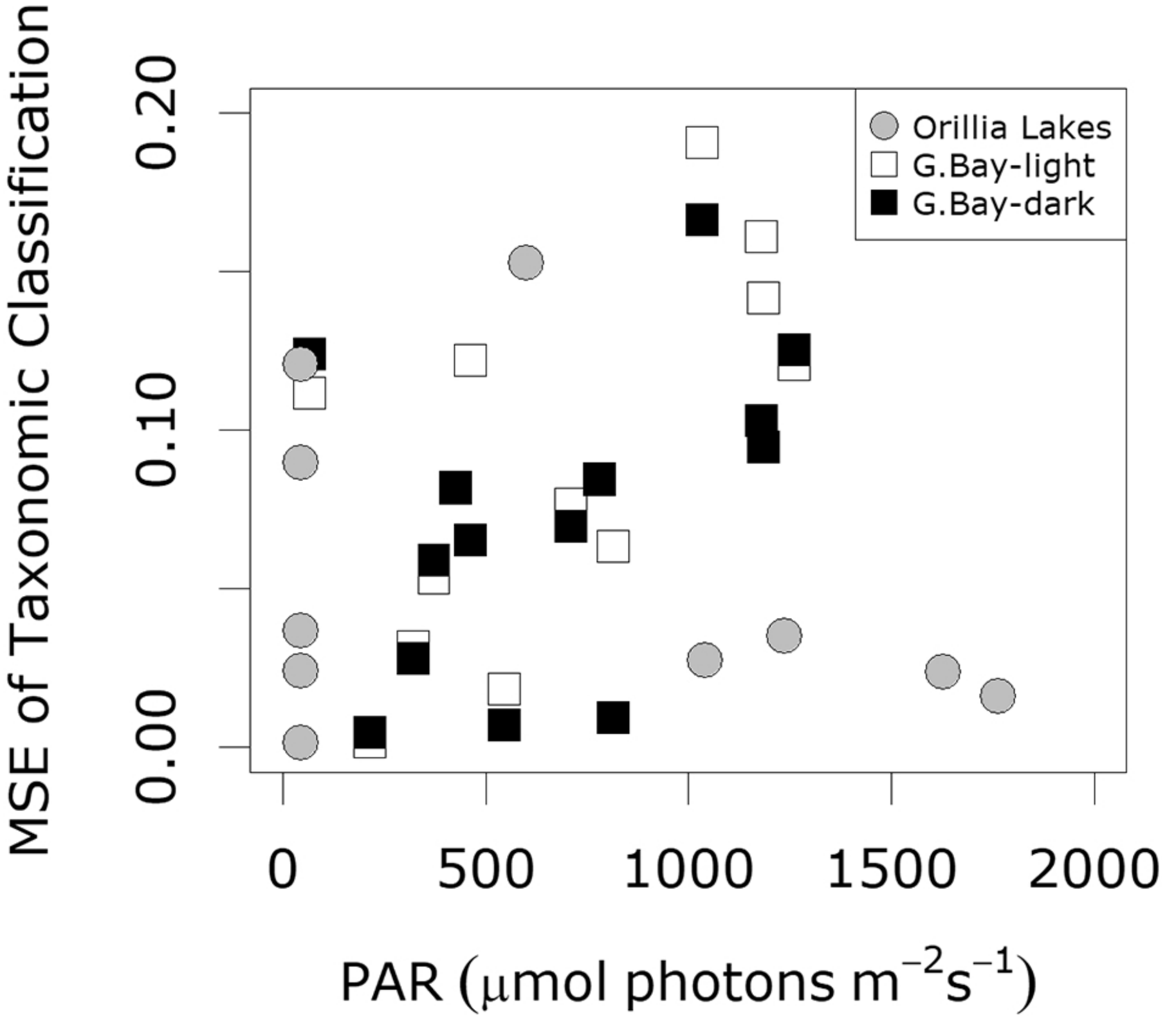
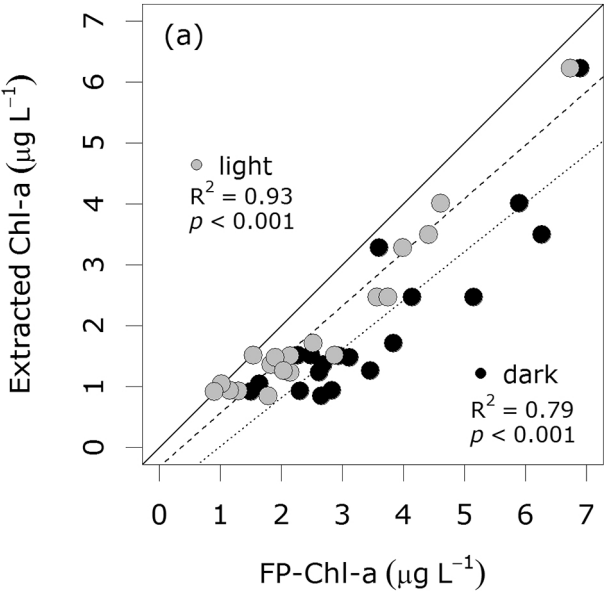

MSE of Taxonomic Classification
0.00
0.10
0.20
0
500
1000
1500
2000
PAR (μmol photons m^{-2}s^{-1})
Orillia Lakes
G.Bay-light
G.Bay-dark

Figure 6


(a)
light
R^2 = 0.93
p < 0.001
dark
R^2 = 0.79
p < 0.001
Extracted Chl-a (μg L^{-1})
FP-Chl-a (μg L^{-1})
0
1
2
3
4
5
6
7

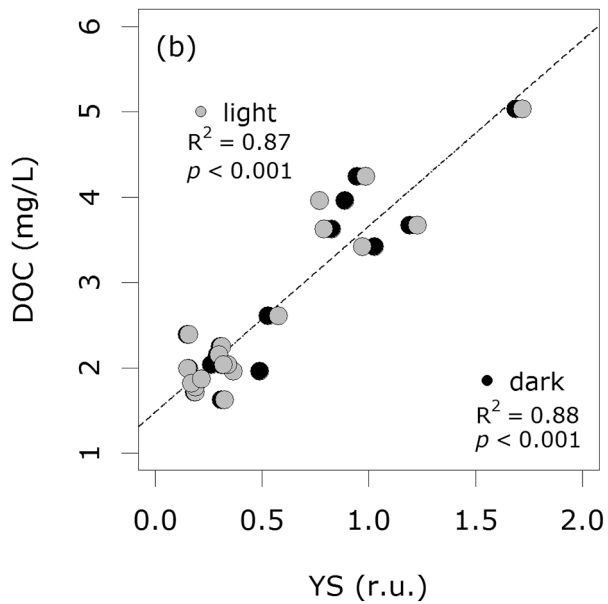

(b)
light
R^2 = 0.87
p < 0.001
dark
R^2 = 0.88
p < 0.001
DOC (mg/L)
YS (r.u.)
1
2
3
4
5
6
0.0
0.5
1.0
1.5
2.0

Figure 7

FP - Dark
FP - Light
Biovolume
Relative Composition
0.0
0.4
0.8
64
214
320
371
424
462
544
710
780
814
1032
1178
1183
1258

Figure 8