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Impact of Regression Model Choice on Light Curve Analysis: Significant Differences without Influence on Biological Interpretation

Abstract

Pulse amplitude modulated chlorophyll fluorometry is a widely used method for assessing photosynthetic performance, providing key parameters such as the maximum electron transport rate (ETR_{max}), light intensity where ETR_{max} is reached (I_m), the photosynthetic efficiency under low light (alpha), and the transition point from light limitation to light saturation (I_k). This study compares the performance of the models by EILERS & PEETERS (1988), PLATT et al. (1980), VOLLENWEIDER (1965) and WALSBY (1997), which are required to determine these parameters. The results show that the Vollenweider model exhibited the lowest deviation from raw data (S_{diff}). No significant differences in ETR_{max} and I_m were found between models, indicating robust determination of these values. However, significant differences in alpha and I_k were model-dependent. Nevertheless, the choice of model had no significant impact on the biological interpretation of the results.

Keywords: pulse amplitude modulated, PAM, photosynthesis, light curve, regression model, model comparison, WALZ

1 Introduction

Measurements of light curves using pulse amplitude modulated (PAM) chlorphyll fluorometry are a widely used tool for characterizing photosynthetic performance (SCHREIBER 2004). These measurements allow the determination of parameters such as the maximum electron transport rate (ETR_{max}), the photosynthetic efficiency under low light (alpha), the transition point from light limitation to light saturation (I_k) and the light intensity where ETR_{max} is reached (I_m) (RALPH & GADEMANN 2005). However, estimating these parameters requires the application of a regression model. Over the past decades, several models have been developed for modeling photosynthesis light curves and adapted for analyzing PAM data. In this study, the focus is on the models by EILERS & PEETERS (1988), PLATT et al. (1980), VOLLENWEIDER (1965), and WALSBY (1997). The determination of the deviation between the measured data and the model prediction allows the assessment of the fit quality (S_{diff}), providing an evaluation of the model accuracy. However, it remains unclear how the choice of model affects the biological interpretability.

The aim of this study is to determine to what extent the results of different models vary, leading to the following hypotheses: 1. The results of the models differ significantly. 2. The choice of model does not affect the biological interpretation. To test these hypotheses, we use a dataset of 111 PAM measurements. First, we examine if the parameter estimates commonly used in photosynthesis research differ significantly between models. Subsequently, we assess whether the significance patterns across four experimental conditions change differently depending on the applied model.

2 Material & Methods

2.1 Sampling and Cultivation

Algal material from 111 individuals was cultivated at the University of Rostock. Cultures were maintained under four different cultivation conditions, referred to as conditions 1, 2, 3, and 4. All cultures were kept for up to six weeks under a light intensity ranging from 93 to 226.5 µmol photons m^{-2} s^{-1}. They differed in cultivation temperature, ranging from 5 °C in condition 1 to 20 °C in condition 4, with condition 2 at 10 °C and condition 3 at 15 °C.

2.2 Messurment of light curves

The samples were dark-acclimated in their cultivation vessels for at least 30 min. A dual PAM chlorophyll fluorometer/absorption spectrometer (DUAL-PAM-100, Heinz Walz GmbH, Germany) was then used to analyze the photosynthetic performance of photosystem II. For the measurement the whole algae were placed in a quartz cuvette with habitat water. Care was taken to ensure that the apical part was in the measurement field. A light curve with 11 irradiation steps (0, 40, 49, 62, 112, 183, 237, 391, 603, 928, 1420 µmol photons m^{-2} s^{-1}) was then recorded. The time set for each step was 60 s up to 62 µmol photons m^{-2} s^{-1} and after 30 s.

2.3 Application of the regression models

For the regression, the models by EILERS & PEETERS (1988), PLATT et al. (1980), VOLLENWEIDER (1965), and WALSBY (1997) were used. The regressions were conducted in R (R CORE TEAM 2024) using the package pam (BÖHM & SCHRAG 2025). Standard start parameters were used. The output of the results was provided in the modified version, as described in the documentation of the package. The influence of photoinhibition was taken into account when determining the parameters. The following parameters were considered: S_{diff}, ETR_{max}, I_m, alpha, I_k.

2.4 Statistics and Data Visualization

The statistical analysis and graphical representation of the data were conducted using R (R CORE TEAM 2024). The following R packages were used: ggplot2 (WICKHAM 2016), ggthemes (ARNOLD 2024), car (FOX et al. 2019), FSA

(OGLE et al. 2025), ggpubr (KASSAMBARA 2023b), multcompView (GRAVES et al. 2024), rstatix (KASSAMBARA 2023a) and patchwork (PEDERSEN 2024). Normality was assessed using Shapiro-Wilk test, followed by Levene's test for homogeneity of variances. Since not all groups were normally distributed and/or homogeneously, the Kruskal-Wallis rank sum test was applied, followed by the Dunn's test as a non-parametric post-hoc test with Bonferroni-adjusted p-values. Results were considered significant at $p < 0.05$.

3 Results

The results indicate that the Vollenweider model exhibited the lowest S_{diff}, whereas the highest S_{diff} was observed for the Platt and Walsby models (Fig. 1A). No significant differences were found between the models for ETR_{max} and I_m (Fig. 1B and Fig. 1C). For alpha, significantly lower values were obtained with the Vollenweider and Eilers & Peeters models compared to Walsby model, while the Vollenweider model also yielded significantly lower values than the Platt model (Fig. 1D). Regarding I_k, significant differences were found among all models, with the highest values obtained in the Vollenweider model and the lowest in the Walsby model (Fig. 1E).

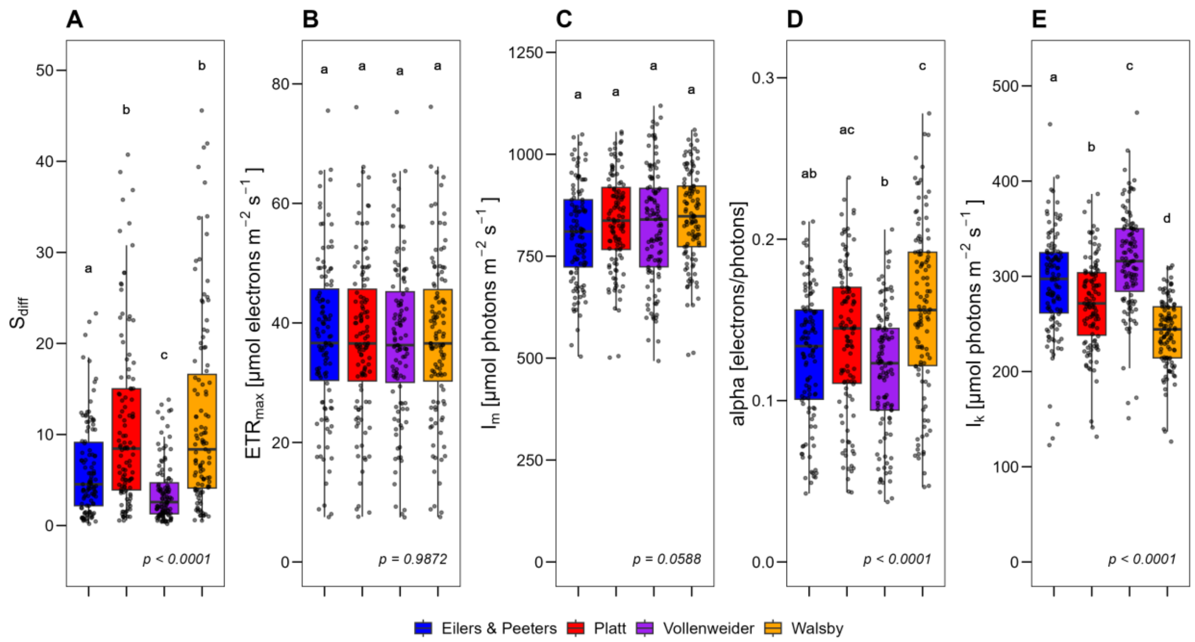

Fig. 1 Comparison of models across different parameters: A) S_{diff}; B) ETR_{max}; C) I_m; D) alpha; E) I_k. The box plots represent the 25th to 75th percentiles, with black lines indicating the median. Whiskers extend to 1.5 times the interquartile range. Semi-transparent black dots represent individual data points. Data points beyond the whiskers represent outliers. Kruskal-Wallis rank sum test results are shown in the bottom right of each diagram. Identical letters indicate statistically similar groups within the same diagram.

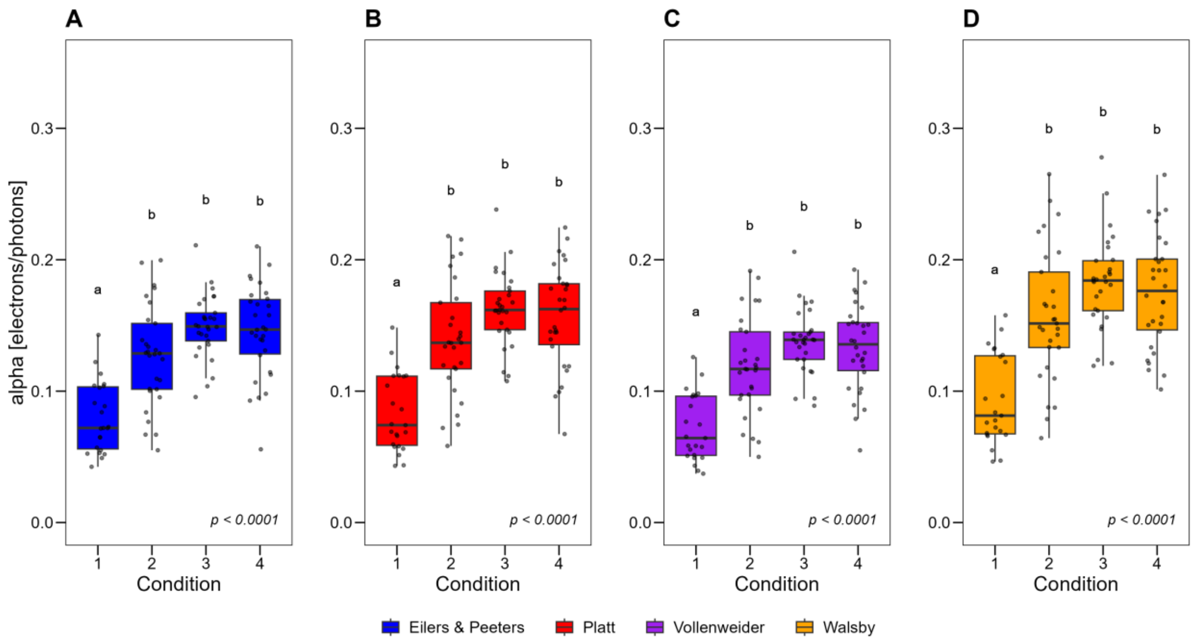

Fig. 2 Comparison of models for the paramter alpha under different experimental conditions: A) Eilers & Peeters; B) Platt; C) Vollenweider; D) Walsby. The box plots represent the 25th to 75th percentiles, with black lines indicating the median. Whiskers extend to 1.5 times the interquartile range. Semi-transparent black dots represent individual data points. Data points beyond the whiskers represent outliers. Kruskal-Wallis rank sum test results are shown in the bottom right of each diagram. Identical letters indicate statistically similar groups within the same diagram.

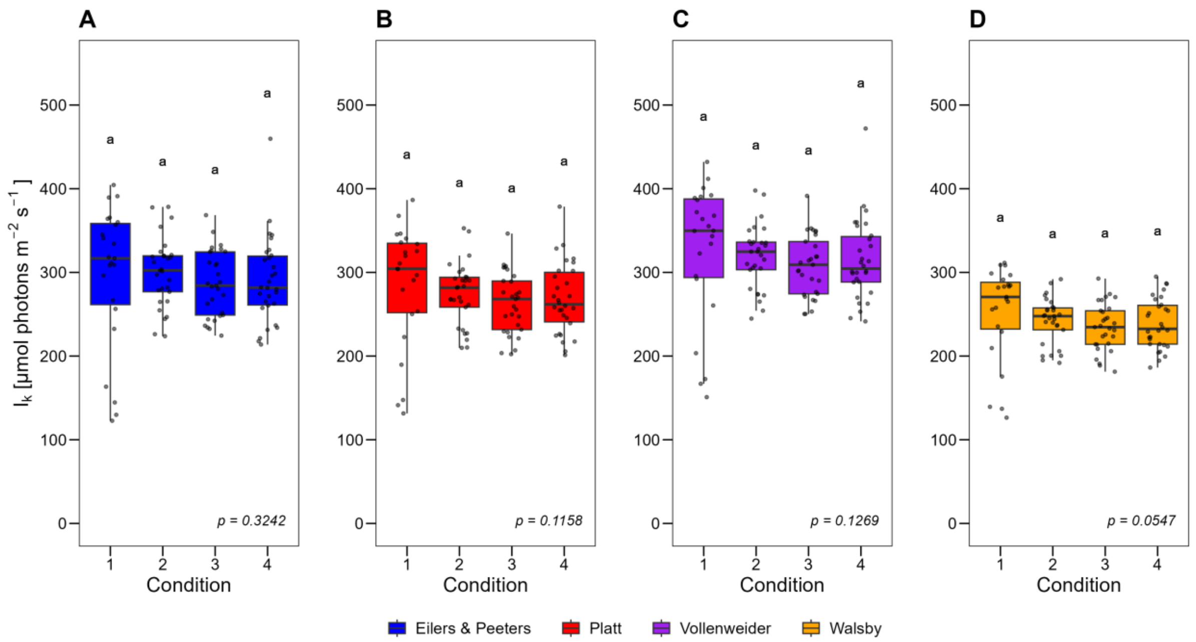

Fig. 3 Comparison of models for the paramter I_k under different experimental conditions: A) Eilers & Peeters; B) Platt; C) Vollenweider; D) Walsby. The box plots represent the 25th to 75th percentiles, with black lines indicating the median. Whiskers extend to 1.5 times the interquartile range. Semi-transparent black dots represent individual data points. Data points beyond the whiskers represent outliers. Kruskal-Wallis rank sum test results are shown in the bottom right of each diagram. Identical letters indicate statistically similar groups within the same diagram.

A detailed analysis of the parameter alpha revealed that all models exhibited the lowest alpha median values under condition 1 and the highest median values under conditions 3 and 4, with condition 2 falling in between. However, a significant difference was only detected between condition 1 and conditions 2, 3, and 4. No shift in significance across models was observed (Fig. 2A–D). For the parameter I_k, the highest median values were generally observed under condition 1, with a decreasing trend toward condition 4. However, this trend was not significant in any of the models (Fig. 3A–D).

4 Discussion

The statistical analysis of the models revealed that the Vollenweider model exhibited the significantly lowest S_{diff} in our dataset. This result is unsurprising, as it is the only model incorporating four variables (VOLLENWEIDER 1965). This allows for a more accurate fit compared to models with only three variables, such as those by EILERS & PEETERS (1988), PLATT et al. (1980), and WALSBY (1997).

The absence of significant differences in ETR_{max} and I_m suggests that the determination of the maximum point of the light curve is robust and consistent across models. Therefore, these parameters are theoretically comparable between different models. In contrast, significant differences were observed for alpha and I_k between the models. Consequently, comparisons of experimental groups for these parameters, when derived from different models, should be avoided. Such comparisons could lead to erroneous significant differences driven by model-specific variations rather than biological or environmental factors. Moreover, this highlights the challenges in precisely determining alpha and I_k. For I_k, it is generally assumed that organisms, after acclimation, adopt an I_k value that approximate reflects the average light conditions of their habitat, although this may not be exact in the field due to constantly fluctuating light conditions (SAKSHAUG et al. 1997). When interpreting whether this applies to a specific experimental group, a certain level of uncertainty should always be considered. Thus, the hypothesis that the results of the models differ significantly can be confirmed, although not for all parameters.

Furthermore, no significant shifts were observed for alpha and I_k between experimental conditions depending on the model. Therefore, the biological validity of whether there is a significant difference between experimental conditions was not influenced by the choice of model. The hypothesis that the choice of model does not affect the biological interpretation can be confirmed.

Zusammenfassung

Die pulsamplitudenmodulierte (PAM) Chlorophyll-Fluorometrie ist eine weit verbreitete Methode zur Charakterisierung und Bewertung der photosynthetischen Leistungsfähigkeit. Sie liefert zentrale Parameter wie die maximale Elektronentransportrate (ETR_{max}), die Lichtintensität, bei der ETR_{max} erreicht wird (I_m), die photosynthetische Effizienz bei geringer Lichtintensität (alpha) sowie den Übergangspunkt von lichtlimitierter zu lichtgesättigter Photosynthese (I_k). In dieser Studie werden die Modelle von EILERS & PEETERS (1988), PLATT et al. (1980), VOLLENWEIDER (1965) und WALSBY (1997) verglichen, die zur Bestimmung dieser

Parameter verwendet werden. Die Ergebnisse zeigen, dass das Modell von Vollenweider die geringste Abweichung von den Rohdaten (S_{diff}) aufwies. Hinsichtlich ETR_{max} und I_m wurden keine signifikanten Unterschiede zwischen den Modellen festgestellt, was auf eine robuste Bestimmung dieser Werte hinweist. Im Gegensatz dazu wiesen alpha und I_k modellabhängige signifikante Unterschiede auf. Dennoch hatte die Wahl des Modells keinen signifikanten Einfluss auf die biologische Interpretation der Ergebnisse.

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