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1 A model for describing the light response of the non-photochemical quenching of chlorophyll
2 fluorescence

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22

23 Abstract

24

25 The operation of photosynthetic energy-dissipating processes is commonly characterised by
26 measuring the light response of the non-photochemical quenching (NPQ) of chlorophyll
27 fluorescence, or NPQ vs. *E* curves. This study proposes a mathematical model for the
28 quantitative description of the generic NPQ vs. *E* curve. The model is an adaptation of the Hill
29 equation and is based on the close dependence of NPQ on the xanthophyll cycle (XC). The
30 model was tested on NPQ vs. *E* curves measured in the plant *Arabidopsis thaliana* and the
31 diatom *Nitzschia palea*, representing the two main types of XC, the violaxanthin-
32 antheraxanthin-zeaxanthin (VAZ) type and the diadinoxanthin-diatoxanthin (DD-DT) type,
33 respectively. The model was also fitted to a large number of published light curves, covering the
34 widest possible range of XC types, taxa, growth conditions, and experimental protocol of curve
35 generation. The model provided a very good fit to experimental and published data, coping with
36 the large variability in curve characteristics. The model was further used to quantitatively
37 compare the light responses of NPQ and of PSII electron transport rate, ETR, through the use of
38 indices combining parameters of the models describing the two types of light-response curves.
39 Their application to experimental and published data showed a systematic large delay of the
40 build-up of NPQ relatively to the saturation of photochemistry. It was found that when ETR
41 reaches saturation, NPQ is on average still below one fifth of its maximum attainable level,
42 which is only reached at irradiances about three times higher. It was also found that organisms
43 having the DD-DT type of XC appeared to be able to start operating the XC at lower irradiances
44 than those of the VAZ type.

45

46

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48 Keyword index: Chlorophyll fluorescence; modelling; non-photochemical quenching;

49 photoacclimation; photoprotection; xanthophyll cycle

50

51 **Introduction**

52
53 Photosynthesis requires a balance between maximizing light absorption and minimizing
54 damages caused by excessively absorbed light energy. Under natural conditions, exposure to
55 sunlight involves unavoidable risks to the photosynthetic apparatus due to the formation of
56 reactive oxygen species of photosynthetic origin produced under excess light (Osmond et al.
57 1997; Ort 2001; Demmig-Adams and Adams 2006). The sustainment of prolonged
58 photosynthetic activity under high solar irradiances is ensured by the regulation of the
59 repartition of absorbed light energy between photochemistry and energy-dissipating pathways.
60 The latter function as photoprotective process against permanent damages to the photosynthetic
61 apparatus, or photoinhibition, and are therefore of considerable interest for understanding the
62 resistance of photoautotrophs to environmental stress (Demmig-Adams and Adams 2006;
63 Lavaud 2007; Li et al. 2009).

64 One of the most important photoprotective processes is the non-photochemical
65 quenching (NPQ). NPQ groups several pathways among which the thermal dissipation of excess
66 energy (or q_E , the energy-dependent quenching, Müller et al., 2001) is considered as the most
67 important. q_E takes place in the light-harvesting antenna of the photosystem II (PS II). It relies
68 on the presence of specific PS II antenna proteins (PsbS in plants, Li et al. 2000; LhcSR in
69 green microalgae, Peers et al. 2009; LhcX in diatoms, Bailleul et al. 2010), the building of a
70 transthylakoid proton gradient (ΔpH), and the subsequent enzyme-mediated operation (de-
71 epoxidation) of the xanthophyll cycle (XC) (Goss and Jakob, 2010). The XC produces de-
72 epoxidised forms of xanthophylls which are essential regulatory partners of q_E , especially in
73 some groups of microalgae (like the diatoms) (Lavaud, 2007; Li et al. 2009; Goss and Jakob
74 2010). In plants, green algae and some Heterokontophyta (brown algae, Chrysophyceae), the
75 XC is based on the reversible conversion of pigment violaxanthin (Vx) to the q_E -involved
76 zeaxanthin (Zx), passing through the intermediate form antheraxanthin (Ax) (VAZ type of XC);
77 in other algal groups, like the diatoms, dinoflagellates, Xanthophyceae and Haptophyta, the XC
78 consists in the conversion between only two pigments, diadinoxanthin (DD) and the q_E -involved

79 diatoxanthin (DT) (DD-DT type of cycle) (Olaizola and Yamamoto 1994; Lavaud et al. 2004;
80 Lavaud 2007). In phycobilisome-containing organisms, there is no XC (cyanobacteria) or its
81 presence is uncertain (red algae) (Goss and Jakob 2010).

82 The operation of NPQ processes is usually quantified using variable chlorophyll
83 fluorescence (Pulse Amplitude Modulation fluorometry, PAM), by calculating the fluorescence
84 index NPQ, which is based on the relative difference between the maximum fluorescence
85 measured in the dark-adapted state, F_m , and upon exposure to light, F_m' (see Table 1 for
86 notation):

87

$$88 \quad \text{NPQ} = \frac{F_m - F_m'}{F_m'} \quad (1)$$

89

90 This index is a rearrangement of the Stern-Volmer equation and reflects the assumption that the
91 reciprocal of fluorescence yield is proportional to the Zx or DT concentration (Bilger and
92 Björkman 1990; Lavaud et al. 2002; Baker and Oxborough 2004). The NPQ index has been
93 routinely used to quantify the operation of photoprotective processes as well as the extent of
94 photoinhibitory damages. Under experimental conditions allowing to assume that the prevailing
95 processes causing the quenching of fluorescence are of photoprotective nature, NPQ has been
96 used as a measure of the overall photoprotective capacity of the photosynthetic apparatus (e.g.
97 Dimier et al. 2007b; Lavaud et al. 2007).

98 One common way to characterise the operation of photoprotective processes or the
99 susceptibility to photoinhibition is to quantify the light response of NPQ. This is done by
100 constructing NPQ vs. E curves that record the development of NPQ with increasing incident
101 irradiance. These curves are analogous to the more frequently measured light-response curves of
102 the PSII electron transport rate (ETR) in the sense that they represent the variation of steady
103 state photosynthetic activity between different light levels, thus not informing on the kinetics of
104 NPQ generation, but on the NPQ attainable under each irradiance. The shape of NPQ vs. E
105 curve varies widely, both regarding its overall shape as well as the absolute NPQ values

106 reached. Typically, NPQ increases monotonically with irradiance, varying from zero (measured
107 in darkness) to maximum values that vary greatly with taxonomical groups, physiological state,
108 environmental constraints, or light levels applied. The curve presents a variable degree of
109 sigmoidicity, ranging from cases when NPQ starts to increase steeply from the lowest light
110 levels, following a simple saturation-like pattern (no sigmoidicity), to cases when NPQ remains
111 close to zero for a range of low light levels, then showing an abrupt increase only for
112 intermediate irradiances before stabilizing at maximum values (highly sigmoid). Often,
113 although the stabilization of NPQ is evident, a constant value is not reached within the range of
114 irradiances applied and the maximum attainable NPQ cannot be estimated.

115 Light-response curves of NPQ have been analysed and interpreted on the basis of
116 arbitrarily chosen features, by qualitatively describing its shape (e.g. low or high sigmoidicity),
117 or by selecting NPQ values reached at particular E levels (e.g. NPQ at maximum applied E).
118 The absence of an adequate descriptive model impedes the characterization of the curve along
119 the whole range of irradiances applied. Also, the lack of a commonly-used set of descriptive
120 parameters makes it difficult to compare curves measured in different studies or experimental
121 conditions or taxonomic groups.

122 The present study proposes a simple mathematical model for the quantitative
123 description of the generic NPQ vs. E curve, by means of the estimation of a small number of
124 physiologically-meaningful parameters. The model is based on the close, but not absolute,
125 dependence of NPQ on the operation of the XC, through the light-induced de-epoxidation of PS
126 II antenna pigments Vx or DD. To illustrate the main features and variability of the NPQ vs. E
127 curve, as well as the interpretation of model parameters, light-response curves were measured
128 on a plant (*Arabidopsis thaliana* (L.); VAZ type XC) and a diatom (*Nitzschia palea* (Kütz) W.
129 Smith; DD-DT type XC) grown under experimental conditions expected to induce a large
130 variability in curve shape and NPQ absolute values. The adequacy of the model was further
131 tested by fitting it to published NPQ vs. E curves measured on a large variety of photosynthetic
132 organisms and experimental conditions, and covering the widest possible range of NPQ values
133 and curve shapes. The usefulness of the model was also illustrated by exploring the relationship

134 between NPQ vs. *E* curves and the photoacclimation status, characterized by light-response
135 curves of the PSII electron transport rate, ETR.

136

137

138 **Materials and Methods**

139

140 Model rationale

141

142 The model proposed to describe the light-response curve of NPQ is an adaptation of the Hill
143 equation, originally derived in the context of ligand binding to macromolecules with multiple
144 binding sites. This equation describes the variation of the number of filled binding sites with the
145 increase in ligand concentration, and is routinely used to characterise the cooperativity of
146 enzymatic reactions (Voet and Voet 1990). The use of the Hill equation for modelling the NPQ
147 vs. *E* curve is based on the main assumption that NPQ is mostly due to the operation of the XC
148 coupled to the build-up of the transthylakoid ΔpH , or energy-dependent quenching (q_E ; Müller
149 et al. 2001), and that the other potential components of NPQ, the quenching due to state
150 transitions (q_T) or to photoinhibition (q_I) are not expected to significantly affect the NPQ vs. *E*
151 curves (see Discussion). The analogy underlying this rationale is to consider the epoxidized XC
152 pigments Vx and DD as representing the macromolecule of the Hill model, and the
153 transthylakoidal ΔpH -dependent protonation of specific light-harvesting complex (LHC)
154 antenna sites as corresponding to the ligand concentration that bind to their multiple binding
155 sites. At steady state, each irradiance level corresponds to a stable transthylakoidal ΔpH and
156 resulting Vx or DD de-epoxidation and activation through protonation of LHC sites (the so-
157 called ‘activation’ of Zx and DT; Horton et al. 2000; Goss et al. 2006; Lavaud and Kroth 2006;
158 Horton et al. 2008), and thus to a defined NPQ value. The NPQ vs. *E* curve is thus proposed to
159 be described by the equation:

160

161
$$\text{NPQ} (E) = \text{NPQ}_m \frac{E^n}{E_{50}^n + E^n} \quad (2)$$

162

163 where NPQ_m is the maximum NPQ value reached during the light curve, E_{50} is the irradiance
 164 level for which NPQ attains 50% of NPQ_m , and n is the Hill coefficient, characterizing the
 165 sigmoidicity of the curve. Whilst the parameters E_{50} and n are directly adopted from the Hill
 166 equation (originally having only these two parameters), a third parameter, NPQ_m , had to be
 167 considered to account for the variability in the absolute values of NPQ. Under the analogy with
 168 the ligand binding context of the Hill equation, $\text{NPQ}(E)$ is considered to represent the fraction
 169 of Vx or DD molecules de-epoxidized into Zx or DT and which have been ‘activated’. As such,
 170 E_{50} represents the irradiance level necessary to de-epoxidize and ‘activate’ 50% of convertible
 171 Vx or DD pool (ligand concentration resulting in half occupation of binding sites) necessary to
 172 reach the maximal NPQ level, NPQ_m . Of potential importance for the characterization of the
 173 physiological processes underlying the NPQ vs. E curve is the sigmoidicity parameter n , which
 174 measures the type and extent of the reaction cooperativity. In the case of $n < 1$, the curve
 175 displays a saturation-like increase asymptotically towards NPQ_m , indicating the presence of a
 176 negatively cooperative reaction; if $n > 1$, the curve starts to present a sigmoidal shape, the
 177 sigmoidicity increasing with n , indicating a positively cooperative, or allosteric, reaction; if n
 178 =1, the model is reduced to the Michaelis-Menten equation, indicative of a non-cooperative
 179 reaction.

180

181 Measurement of light-response curves

182

183 To test the adequacy of the model, Eq. 2 was first fit to NPQ vs. E curves generated for
 184 organisms representative of the two types of XC, a plant (*A. thaliana*; VAZ type of XC) and a
 185 diatom (*N. palea*; DD-DT type of XC), grown under light conditions expected to induce large
 186 variations in NPQ absolute values and in the shape of the NPQ light-response curve. Plants of
 187 *A. thaliana* (ecotype Columbia) were grown under controlled growth chamber conditions for 4–

188 5 weeks under a 16:8 h light/dark cycle and 70% relative humidity. The light/dark temperatures
 189 were 22/20 °C. Three irradiance levels were applied: 10 ('low light', LL), 75 ('moderate light',
 190 ML) and 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ('high light', HL). The diatom *Nitzschia palea* (Kütz.) W.
 191 Smith (collection of the Department of Biology, University of Aveiro) was grown
 192 photoautotrophically in unialgal semi-continuous batch 100 mL cultures in sterile natural
 193 seawater enriched with f/2 nutrients (Guillard and Ryther 1962). Cultures were grown at 20 °C,
 194 under 20 (LL), 100 (ML) and 400 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (HL) in a 12:12 h light/dark cycle. Cells
 195 were harvested by centrifugation ($3000 \times g$, 10 min) during the exponential phase of growth,
 196 and were resuspended in fresh growth medium supplemented with NaHCO_3 to a final
 197 concentration $> 10 \mu\text{g Chl } a \text{ mL}^{-1}$.

198 Light-response curves of NPQ and ETR were generated by exposing the samples to 7-
 199 12 levels of actinic light, up to $920 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Samples were dark-adapted for 30 min before
 200 the start of the light curve to allow determination of fluorescence levels F_o (minimum
 201 fluorescence) and F_m , required for the calculation of NPQ (Eq. 1). Samples were light-activated
 202 before the start of each light-response curve, through exposure to low light of $54 \mu\text{mol photons}$
 203 $\text{m}^{-2} \text{ s}^{-1}$ until a steady state in fluorescence was reached (minimum 15 min). Under each light
 204 level, a saturation pulse (0.8 s for *A. thaliana* and 0.6 s for *N. palea*) was applied and
 205 fluorescence levels F_s (steady state fluorescence) and F_m' were recorded and used to calculate
 206 NPQ (Eq. 1) and ETR, using (Genty et al. 1989):

207

$$208 \quad \text{ETR} = E \frac{F_m' - F_s}{F_m'} \quad (3)$$

209

210 A different sample (plant leaf or algal culture aliquot) was used for measuring NPQ and ETR
 211 under each light level. Three replicated measurements were made for each light level. Light-
 212 response curves of ETR were described by fitting the model of Eilers and Peeters (1988), and by
 213 estimating the parameters α (the initial slope of the curve), ETR_m (maximum ETR) and E_k (the
 214 light-saturation parameter). The parameter E_k is commonly interpreted as a measure of the light

215 level to which a sample is acclimated to, and commonly used to characterise its
216 photoacclimation status (Behrenfeld et al. 2004).

217 Chlorophyll fluorescence yield was measured using a PAM fluorometer comprising a
218 computer-operated PAM-Control Unit (Walz) and a WATER-EDF-Universal emitter-detector
219 unit (Gademann Instruments GmbH, Germany), using a modulated blue light (LED-lamp
220 peaking at 450 nm, half-bandwidth of 20 nm) as source for measuring, actinic, and saturating
221 light (Cruz and Serôdio 2008). Fluorescence was measured using a 6 mm-diameter Fluid Light
222 Guide fiberoptics. In the case of *N. palea*, the fiberoptics was connected to a fluorescence
223 cuvette (KS-101, Walz, Effeltrich, Germany).

224

225 Published light-response curves

226

227 The model was also fitted to published NPQ vs. *E* curves, covering a wide range of taxonomic
228 groups (higher plants, mosses, green algae, diatoms, dinoflagellates, others), XC types (VAZ,
229 DD-DT, absence of XC), mutants with variable degrees of impairment of the XC operation,
230 habitats (land and aquatic higher plants; planktonic and benthic microalgae), growth conditions
231 (e.g. low and high light), physiological state (heat stress or XC inhibitors) and fluorescence
232 light-response curve measuring protocols (steady state and rapid light curves, RLCs). Only
233 species with a known functional XC were considered. Studies presenting light curves having
234 less than seven data points were not considered, because of the large errors in the model fitting
235 and parameter estimation. For each study, when three or more light curves were available for
236 each species and experimental treatment, only the two curves presenting the most extreme
237 (higher and lower) NPQ values were used. Detailed information on the data used in the meta-
238 analysis is summarised in Table 3. When available, the corresponding ETR vs. *E* curves were
239 also characterised by fitting the model of Eilers and Peeters (1988) and by estimating its
240 parameters.

241 Some studies have reported the formation of NPQ in the dark (Perkins et al. 2010). This
242 phenomenon was observed on diatoms (Jakob et al. 1999) and brown algae (Mouget and

243 Tremblin 2002), and results in NPQ vs. *E* curves showing a distinctive bi-phasic pattern, with
244 NPQ decreasing from initial high values measured in the dark to a minimum under low to
245 moderate light levels (e.g. Geel et al. 1997; Serôdio et al. 2006). Being relevant only for a
246 limited number of taxonomic groups and physiological conditions, this type of NPQ light-
247 response curve was not considered in the present study.

248

249 Model fitting and parameter estimation

250

251 The light-response curve models were fitted using a procedure written in Microsoft Visual Basic
252 and based on Microsoft Excel Solver. Model parameters were estimated iteratively by
253 minimizing a least-squares function, forward differencing, and the default quasi-Newton search
254 method. The model can be easily fitted using commonly available software packages. On
255 preliminary tests, this method was compared with the fitting procedures implemented in
256 Sigmaplot 9.0 (Systat Software, Inc., San Jose, USA) and Statistica 8.0 (Statsoft, Inc., Tulsa,
257 USA) and no significant differences were found between the estimates of model parameters.

258 The standard errors of the parameter estimates were calculated following Ritchie (2008)
259 and are asymptotic standard errors. For nonlinear models such as the one here tested, asymptotic
260 standard errors may not be adequate, as they possibly underestimate the actual parameter
261 uncertainty and cannot evaluate the eventual asymmetry of the confidence regions of estimated
262 parameters (Johnson 2008). A number of alternative approaches exist, the best being based on
263 Monte Carlo methods, such as the Bootstrap (Press et al. 1996; Johnson 2008). However, the
264 benefits of pursuing alternative methods, requiring substantially more computation time, depend
265 on the magnitude of the uncertainty associated to parameter estimation. In this study, the use of
266 asymptotic standard errors was justified by the finding that they were on average relatively low,
267 in a majority of cases below 10% of parameter estimates (see below, Results), in which case
268 they can be considered acceptable (Tellinghuisen 2008).

269

270 **Results**

271
272 Model fitting to NPQ vs. *E* curves
273
274 The light-response curves of NPQ measured in the plant *A. thaliana* and in the diatom *N. palea*
275 acclimated to different growth light conditions showed a wide range of curve shapes, varying
276 between two main types: curves with low sigmoidicity, presenting a simple saturation-like
277 pattern (e.g. Fig. 1a, HL), and curves with high sigmoidicity, presenting an initial period of low
278 values, followed by a phase of rapid increase leading to a stage of plateau of maximum values
279 (e.g. Fig. 2a, HL). In all cases, the model provided an excellent fitting to the experimental data
280 throughout the whole range of light levels explaining always more than 99.1% of the data
281 variability (Table 2). Although the residuals (Figs. 1b, 2b) showed in some cases a clearly non-
282 random pattern of variation, values were typically low, between $\pm 5\%$. The fitting of the model
283 resulted in a large variation of model parameters with species and growth light conditions,
284 following some consistent trends. Both for *A. thaliana* and *N. palea*, the increase in growth light
285 resulted in an increase in NPQ_m (Fig. 1a, 2a; Table 2). This increase in NPQ_m could be detected
286 despite the fact that in most cases the curves did not reach their maximum values within the
287 range of irradiances applied (e.g. Fig. 1a). Another effect of increasing growth light was the
288 increase in the irradiance level for which the curves reached maximum values. For LL-grown
289 samples, maximum NPQ was reached for lower irradiances, with the curves showing an overall
290 lower sigmoidicity, while for HL-grown samples, curves reached saturation at much higher light
291 levels. This trend could be well described by model parameter E_{50} , which increased with growth
292 light in both species (Table 2).

293 The most noticeable difference between the NPQ light-response curves of *A. thaliana*
294 and *N. palea* was the apparent curve sigmoidicity, with less sigmoid curves being found for the
295 former and more sigmoid curves (and a larger variation) for the latter. This variation in curve
296 sigmoidicity was well characterized by the model parameter n , which averaged 1.2 for *A.*
297 *thaliana* (varying from 0.98 to 1.44), whilst reaching an average value of 2.11 for *N. palea*

298 (varying from 1.61 to 2.61). However, a different pattern of variation was found for each
299 species, with n decreasing with growth light in *A. thaliana*, and increasing in *N. palea* (Table 2).

300

301 Model fitting to published NPQ vs. E curves

302

303 The compilation of published light-response curves of NPQ allowed gathering a large number
304 of datasets covering a wide range of NPQ absolute values and curve shapes. The model
305 provided a very good fit to the published data, coping with the large diversity in curve
306 characteristics resulting from different combinations of maximum NPQ values attained (NPQ_m),
307 irradiance range of NPQ build-up (E_{50}), and curve sigmoidicity (n) (Table 3). Considering all
308 cases, the model explained more than 96% of data variability, independently of type of XC (and
309 XC impaired mutants), species, growth conditions, and experimental protocol used for the
310 generation of light curves. A summary of the meta-analysis carried out on the data set of
311 published NPQ vs. E curves is presented in Table 4. NPQ_m was the most variable parameter
312 (c.v. 112.5%), mostly responding to growth conditions, averaging 2.89 but reaching maximum
313 values above 9 for diatoms and even higher for some mosses (Table 3). E_{50} ranged from 30-40
314 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ to above 3500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, in all cases attaining values well above growth
315 irradiances (see below). However, with few exception (the diatoms *Skeletonema costatum* and
316 *Phaeocystis antarctica*, and the high light-grown *A. thaliana*), all values of E_{50} above 1200
317 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ were obtained for *A. thaliana* mutants with impaired operation of the XC (Table 3).
318 n was the least variable parameter (c.v. 50.8%), meaning that the curve shape remained
319 relatively unaltered, and that the variability in the NPQ vs. E curves was mainly due to changes
320 in NPQ maximum values and in NPQ onset along the irradiance range. n averaged 1.7,
321 corresponding to a noticeable degree of sigmoidicity, although values around 1.0 (no
322 sigmoidicity) or slightly lower (mostly mutants with impaired XC) as well as above 3.0 (very
323 high sigmoidicity) were also found (Table 3).

324 Despite the low number of data points forming most NPQ vs. E curves, standard errors
325 of parameter estimates were on average relatively low (15.6, 27.3 and 12.2% of estimates for

326 NPQ_m, E_{50} and n , respectively). However, standard errors were typically much higher in the
327 cases when XC was impaired (24.5, 43.9 and 13.5% for NPQ_m, E_{50} and n , respectively),
328 indicating that a high level of precision in the estimation of model parameters can be expected
329 for samples under natural conditions.

330 When comparing different XC types, no clear differences were found between model
331 parameters, due to the large overlap of their range of variation. Model parameters appeared to
332 depend mostly on growth conditions, as larger variations could be found within the same
333 species than amongst different taxonomic groups. The model also described very well the light-
334 response curves of NPQ for composite samples (phytoplankton, microphytobenthos). Model
335 parameters varied independently from each other, as no significant correlations were found
336 between NPQ_m, E_{50} or n , either when considering all data pooled together, or when considering
337 for separate for each XC type.

338 Considering the published data, the effects of growth light levels on model parameters
339 were identical to those described for the experimental data of this study. When compared to
340 lower light conditions, NPQ light-response curves of samples acclimated to high light showed
341 higher values of NPQ_m (Bilger and Bjorkman 1990; Burritt and MacKenzie 2003; Müller et al.
342 2004; Ralph and Gademann 2005; Rodríguez-Calcerrada et al. 2007; Cruz and Serôdio 2008)
343 and, with the exception of Ralph and Gademann (2005), all these studies showed a similar
344 increase in E_{50} with growth light. The model also fitted very well the light-response curves of
345 NPQ with impaired functioning of the XC, illustrating the usefulness of estimating model
346 parameters to characterize quantitatively the effects on NPQ of inhibitors (e.g. Bilger and
347 Bjorkman 1990), mutations (e.g. Niyogi et al. 1998), or changes in temperature (Ralph et al.
348 2001).

349 Finally, the model was also found to be adequate to describe NPQ vs. E curves
350 generated under non-steady state conditions, i.e. derived from RLCs. The comparison of the
351 model parameters estimated for light curves of different light steps showed the same pattern on
352 VAZ and DD-DT samples, with shorter light steps resulting in lower NPQ_m and higher E_{50} ,
353 whilst n not showing any consistent trend (Ralph and Gademann 2005; Perkins et al. 2006).

354

355 Relationship between NPQ and ETR light-response curves

356

357 To illustrate the application of the model to evaluate to what extent the NPQ response to light is
358 related to the photoacclimation status, the light levels required for induction of NPQ and for
359 photochemistry or ETR saturation were compared. A simple form of achieving this is by the
360 direct comparison of model parameters E_{50} and E_k estimated for the two types of light-response
361 curves. These parameters have the advantage of being measured in the same scale (PAR
362 irradiance, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and of being independent of the units used to measure
363 photosynthetic rates, ETR or NPQ, which can vary with the method in use (the case of
364 photosynthesis) or instrument settings (the case of ETR).

365 For the experimental data obtained in this study, the increase in growth irradiance
366 induced a substantial change in the photoacclimation status, noticeable by a clear change in the
367 ETR vs E curves. As a result, the increase observed for E_{50} was followed by a similarly large
368 increase in E_k , (due to the decrease of α and the increase of ETR_m), both in *A. thaliana* (Fig. 3)
369 and in *N. palea* (Fig. 4). However, E_{50} was found to be in all cases higher than E_k , by 3.7 and
370 2.5 times, on average, for *A. thaliana* and *N. palea*, respectively (2.90 for the whole data). This
371 result indicates that the light-induced build-up of substantial values of NPQ started only after
372 linear electron transport reached near saturation. Furthermore, it shows that a large fraction of
373 E_{50} variability was related to variations in the photoacclimation status due to different growth
374 light conditions, as E_{50} increased linearly with E_k , both for the plant as for the diatom (although
375 the correlation was not statistical significant, but a clear linear trend is obvious; Fig. 5). This
376 analysis also showed that the slope of the regression line of E_{50} on E_k was higher for *A. thaliana*
377 than for *N. palea*, indicating that, for the same photoacclimation status (the same E_k) the plant
378 requires a higher light level for NPQ to reach 50% of its maximum capacity. Or, in other words,
379 that NPQ is induced latter in the range of irradiances in the plant than in the diatom.

380 Interestingly, the analysis of the subset of published data presenting both NPQ and ETR
381 light-response curves, showed the same generic trend. When pooling together the published and

382 experimental data for samples with fully operational XC (i.e. excluding mutants or samples
383 treated with XC inhibitors) and E_k estimates based only on steady state light curves (i.e.
384 excluding RLCs as E_k estimation is largely dependent on light step duration; Serôdio et al.
385 2006), the ratio E_{50}/E_k was found to be higher on organisms having a VAZ type of XC and to
386 differ significantly from those having a DD-DT type (3.32 and 2.39, respectively; t -test $P =$
387 0.038; Fig. 6a).

388 Another way to compare the light responses of NPQ and ETR is to calculate the fraction
389 of NPQ that is formed when ETR approaches saturation (i.e. when $E = E_k$), or:

390

$$391 \quad \text{NPQ}_{E_k} = \frac{\text{NPQ}(E_k)}{\text{NPQ}_m} \quad (4)$$

392

393 Low values of NPQ_{E_k} indicate that when photochemistry saturates, NPQ is still not significantly
394 developed (corresponding to high E_{50}/E_k), whilst high values indicate that NPQ responds more
395 promptly to light increase and the approach of ETR saturation (corresponding to low E_{50}/E_k).
396 Considering both published and experimental data (the data subset used above for comparing
397 E_{50}/E_k), NPQ_{E_k} averaged 0.16, and remained below 0.35, indicating that when $E = E_k$, NPQ
398 hardly reached over one third of the maximum attainable level. Confirming the expected
399 inverted relationship between NPQ_{E_k} and E_{50}/E_k , significantly lower values were found for
400 organisms with a VAZ type of XC than for those with the DD-DT type (0.14 and 0.21,
401 respectively; t -test, $P = 0.023$; Fig. 6b).

402

403 Model fitting and parameter estimation

404

405 Despite the usually reduced number of data points in the analyzed NPQ light-response curves
406 (average 9.7, maximum 13), the model proposed in this study was found to be easily fitted to
407 experimental curves, in most cases yielding parameter estimates virtually independently from
408 the start values used in the iterative fitting procedure (although realistic start values allows a

409 more rapid and precise parameter estimation). The main problem found when trying to fit Eq. 2
410 to NPQ vs. E curves occurred when the curves were very close to linear, showing no clear
411 features like sigmoidicity or saturation. This resulted in that a similarly good fit could be
412 reached with different combinations of model parameters values. Still, this occurred only in
413 relatively small number of cases, like the case of the diatom *Skeletonema costatum* (Lavaud et
414 al. 2007) and some *A. thaliana* mutants with severe impairment of XC operation, for which
415 some degree of uncertainty remain associated to the presented parameter estimates.

416 This problem seems to result from the common situation of having light-response
417 curves constructed with the main purpose of characterizing the ETR vs. E curves, NPQ having
418 only a secondary interest. As it often happens that the NPQ approaches its maximum for light
419 levels much above the range used for measuring ETR, the NPQ vs. E curve may result truncated
420 and its complete shape may not be available for model fitting. Yet, the relationship established
421 between NPQ and ETR light curves in similar data may be used to impose boundaries to the
422 model parameter estimates, and thus help to reach a single set of meaningful values.

423

424

425 **Discussion**

426

427 Model assumptions

428

429 The model proposed for describing the NPQ vs. E curve is based on the main assumption that
430 NPQ is mostly due to q_E , the ‘energy-quenching’ associated to the build-up of the transthylakoid
431 ΔpH and the operation of the XC. This assumption is generally supported by the finding of
432 strong linear relationships between NPQ and de-epoxidized xanthophylls in all groups
433 investigated (plants, green algae, diatoms, Chrysophyceae) and under a wide range of
434 experimental conditions.

435 However, besides q_E , NPQ also quantifies the fluorescence quenching caused by state
436 transitions (q_T) or by photoinhibition (q_I) which may potentially significantly affect the NPQ vs.

437 *E* curves. q_T is relevant in phycobilisome-containing organisms (cyanobacteria and red algae)
438 (Campbell et al. 1998; Mullineaux and Emlyn-Jones 2004), although not as much in higher
439 plants and green algae (Pfannschmidt 2005; Eberhard et al. 2008; Ruban and Johnson 2009),
440 and it does not occur for the Heterokontophyta (Lavaud 2007). When occurring, q_T is not
441 significant in light conditions triggering q_E -related NPQ (Mullineaux and Emlyn-Jones 2004;
442 Tikkanen et al. 2006; Ruban and Johnson 2009). With the exception of some land plants
443 (overwintering conifers and tropical evergreen species), q_I origin is not clearly defined and it
444 requires particular conditions such as prolonged environmental stress (Müller et al. 2001;
445 Demmig-Adams and Adams 2006; Horton et al. 2008).

446 The effects of q_T or q_I on the light-response curve of NPQ would likely consist in a
447 general change in curve shape, through the increase of NPQ at low light levels (for q_T) and of
448 NPQ_m (for q_I). However, these components of NPQ may be expected not to significantly affect
449 the NPQ vs. *E* curves measured under the usually applied experimental protocols. This is
450 because the duration of the light steps commonly applied (≤ 2 -3 min, and much shorter in the
451 case of RLCs) correspond to light doses clearly different than those required to induce both q_T
452 or q_I (Campbell et al. 1998; Müller et al. 2001; Demmig-Adams and Adams 2006; Horton et al.
453 2008; Ruban and Johnson 2009).

454 The same reasoning applies to another implicit assumption of the model, that during the
455 generation of a light curve q_E is due solely to the development of the ΔpH and the subsequent
456 de-epoxidation of the present (and susceptible of being de-epoxidized) pool of xanthophylls. In
457 fact, it is well established that exposure to high light can induce the *de novo* synthesis of XC
458 pigments (e.g. Olaizola and Yamamoto 1994; D'Haese et al. 2004; Lavaud et al. 2004;
459 Demmig-Adams and Adams 2006). This process causes the accumulation of de-epoxidized
460 forms, which could result in the linear increase of NPQ in the high-light part of the curve.
461 Again, the *de novo* synthesis requires the prolonged exposure to very high irradiances (e.g.
462 Lavaud et al. 2004; Demmig-Adams and Adams 2006; Lavaud 2007), well above the light
463 doses applied during the construction of NPQ light-response curves.

464 Nevertheless, even if significant q_T , q_I or *de novo* pigment synthesis do occur, the
465 resulting curve may still be of the same general shape and describable by the model. In fact, this
466 study showed that the model fits very well to data obtained in a wide range of experimental
467 conditions, in which the occurrence of any of these processes cannot be completely ruled out
468 (Table 3). In this case, however, care should be taken in interpreting and comparing the model
469 parameters estimated for different species or condition, as they may be affected differently by
470 processes other than the build-up of the ΔpH and the XC. In the case of organisms with
471 impaired operation of the XC (mutants or inhibitor-treated samples), the model was nevertheless
472 shown to be of value, providing a useful quantitative characterization of the light response of
473 NPQ.

474 Another major assumption of the model is that the formation of q_E , through the de-
475 epoxidation of Vx or DD generated by the acidification of the lumen relates to incident
476 irradiance following the generic conditions of application of the Hill equation, i.e. that NPQ can
477 be considered as allosterically regulated by irradiance through the build-up of the
478 transthylakoidal ΔpH . This assumption is partially supported by the finding that, in isolated
479 plant chloroplasts, the regulation of q_E is of allosteric nature, resulting in response curves of q_E
480 as a function of ΔpH of sigmoidal shape, shown to be adequately described by the Hill equation
481 (Pfündel and Dilley 1993; Ruban et al. 2001; Takizawa et al. 2007; Pérez-Bueno et al. 2008).
482 This sigmoidicity is attributed to the protonation of binding sites of the LHC protein *PsbS*
483 induced by lumen acidification, and subsequent conformational changes within the LHC,
484 switching the de-epoxidized xanthophylls to an ‘activated’ state. It is taken as an indication of
485 allosteric regulation of q_E , resulting from proton binding showing positive cooperativity, and the
486 ‘activated’ de-epoxidized xanthophylls Zx (Horton et al. 2000; Horton et al. 2008; Li et al.
487 2009) or DT (Goss et al. 2006; Lavaud and Kroth 2006) acting as a positive effector.

488 The strong physiological basis of the proposed model is likely to explain its success to
489 cope with a large diversity of light-response curve characteristics. Other mathematical models
490 have also been used to successfully describe NPQ vs. *E* curves, namely an exponential
491 saturation function, the Michaelis-Menten function, and a hyperbolic tangent function (Ritchie

492 2008). However, these models have only been tested on a reduced dataset, and on light curves
493 of low sigmoidicity. Interestingly, the model that yielded the better fit to experimental data was
494 the Michaelis-Menten equation (Ritchie 2008), a particular case of the model proposed in the
495 present study.

496

497 Model fitting to NPQ vs. E curves

498

499 The usefulness of the model was demonstrated by the possibility to estimate NPQ_m despite the
500 fact that in most cases the curves did not reach a saturation plateau within the range of
501 irradiances applied (e.g. Fig. 1a). When assuming NPQ to represent mostly q_E , the maximum
502 NPQ reached by a sample quantifies its photoprotective potential via the dissipation of
503 excessive light energy. By applying the model, curves measured under different experimental
504 conditions can be readily compared, and changes in NPQ_m (e.g. following changes in growth
505 light conditions) can be quantified, which would be otherwise impossible. The accurate
506 estimation of NPQ_m is also required for quantifying E_{50} , which, in some cases of strict
507 relationship (e.g. for most Heterokontophyta; Lavaud et al. 2004; Lavaud 2007), is interpretable
508 as the light level corresponding to the de-epoxidation and ‘activation’ of the xanthophyll
509 convertible pool necessary to induce half of the maximal NPQ. The size of the xanthophyll
510 convertible pool can be very different in VAZ and DD-DT organisms varying with species and
511 growth conditions, ranging between 50 and 70% (Lavaud 2007; Goss and Jakob 2010). Again,
512 the fit of Eq. 2 to NPQ vs. E curves provided an adequate form of characterizing how promptly
513 the XC is ΔpH -activated when responding to an increase in incident irradiance.

514 However, while NPQ_m and E_{50} can be grossly estimated by visual inspection of the
515 curves (at least when saturation is reached for the light levels applied), the curve sigmoidicity
516 can hardly be characterized quantitatively without the fit of the model and estimation of n . The
517 sigmoidicity coefficient was found to vary around 1.5-2.0, values corresponding to positive
518 cooperativity and thus indicating the allosteric regulation of NPQ by light. Minimum n values
519 averaged about 1 (reaching significantly lower values only for cases of XC impairment),

520 indicate that negative cooperativity is not involved in the regulation of processes underlying
521 NPQ. These results generally support that the allosteric nature known for the more fundamental
522 regulation of q_E by ΔpH in plants (Ruban et al. 2001; Li et al. 2009) holds for the relationship of
523 NPQ to irradiance in a large diversity of photosynthetic organisms.

524 The Hill coefficient has been interpreted as representing the number of allosteric
525 regulators (D'Haese et al. 2004). Considering the diversity of factors likely to contribute to the
526 NPQ vs. E curve (Demmig-Adams and Adams 2006; Eberhard et al. 2008; Horton et al. 2008;
527 Li et al. 2009), some of them not fully identified in some photosynthetic taxa (Lavaud 2007), n
528 should be considered simply as an empirical coefficient informing on the degree of allostery and
529 serving as a practical descriptor of the curve shape.

530

531 Relationship of NPQ light response to photoacclimation status

532

533 The modelling of the NPQ vs. E curves opens the new possibility of quantitatively comparing
534 the light responses of NPQ and of photochemistry or ETR. Photosynthesis and ETR light-
535 response curves are very commonly used as a form of characterizing the photoacclimation status
536 of photosynthetic organisms, through the estimation of the parameters of a number of available
537 models (Henley 1993; Behrenfeld et al. 2004; Ralph and Gademann 2005; Guarini and Moritz
538 2009; Perkins et al. 2010). By describing the NPQ vs. E curve by a small set of meaningful
539 parameters, the light response of NPQ can be characterized relatively to the one of ETR or
540 photosynthetic rate.

541 This possibility is useful for the definition of the light levels corresponding to the onset
542 of NPQ (e.g. activation of the XC) relatively to the saturation of photochemistry. As the light
543 response of NPQ may depend greatly on the photoacclimation status, the characterization and
544 comparison of NPQ vs. E curves should be preferably normalized to parameters indicative of
545 photochemistry saturation. Furthermore, the comparison of the NPQ and ETR light responses is
546 also of interest because it provides insight on the way an organism combines the responses of
547 photochemistry and of photoprotective processes to changes in ambient light. However, without

548 adequate parameterization of the NPQ light-response, this question can be answered only
549 approximately.

550 Despite its simplicity, the indices E_{50}/E_k and NPQ_{E_k} here proposed provide an efficient
551 form of comparing the light levels for which NPQ reaches significant values and
552 photochemistry reaches saturation. The application of these indices allowed quantifying the
553 delay of the light response of NPQ relatively to the saturation of photochemistry. Under the
554 assumption that NPQ mostly represents q_E , it showed that it takes about three times the
555 irradiance at E_k for half of q_E to develop and that, at E_k , the formation of q_E is still at relatively
556 low levels, providing typically less than one fifth of maximum photoprotective capacity. For the
557 cases when q_E is strictly related to the XC operation (e.g. in most of Heterokontophyta; Lavaud
558 et al. 2004; Lavaud 2007), the values of the indices E_{50}/E_k and NPQ_{E_k} can be interpreted in
559 terms of the de-epoxidation state of the convertible pool of xanthophylls and their ‘activation’
560 upon LHC protonation. Yet, the modelling of the NPQ vs. E curve may allow for the
561 development of other indices that better characterize this relationship.

562 Interestingly, the meta-analysis carried out in this study showed consistent differences
563 between the relationships of NPQ light response to photoacclimation status in organisms with
564 VAZ and DD-DT types of XC. The fact that these differences were found despite the diversity
565 in taxa and growth conditions may indicate that they are due to fundamental differences in the
566 way the two types of organisms cope with high light, especially regarding the underlying q_E
567 mechanism and regulation of the XC, illustrating the advantages provided by the quantitative
568 description of the NPQ vs E curve.

569

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571

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820	Table 1. Notation
821	
822	α – Initial slope of the ETR vs. E curve
823	Ax – Antheraxanthin
824	DD – Diadinoxanthin
825	DT – Diatoxanthin
826	E – PAR irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
827	E_{50} – Irradiance level corresponding to 50% of NPQ_m in a NPQ vs. E curve
828	E_k – Light-saturation parameter of the ETR vs. E curve
829	ETR – PSII relative electron transport rate
830	ETR_m – Maximum ETR in a ETR vs. E curve
831	F_o, F_m – Minimum and maximum fluorescence of a dark-adapted sample
832	F_s, F_m' – Steady state and maximum fluorescence of a light-adapted sample
833	NPQ – Non-photochemical quenching
834	NPQ_m – Maximum NPQ value reached in a NPQ vs. E curve
835	NPQ_{E_k} – Fraction of NPQ_m reached when $E = E_k$
836	n – Sigmoidicity coefficient of the NPQ vs. E curve
837	PSII – Photosystem II
838	RLC – Rapid light-response curve
839	VAZ – Vx-Ax-Zx XC
840	Vx – Violaxanthin
841	XC – Xanthophyll cycle
842	Zx – Zeaxanthin
843	

844
 845 Table 2. Summary of the results of the fitting of the model to NPQ vs. *E* curves measured in *A.*
 846 *thaliana* and *N. palea* grown under low (LL), moderate (ML) and high light (HL). Parameter
 847 estimates \pm one standard error.

		Model parameters			
		NPQ _m	<i>E</i> ₅₀	n	r ²
<i>A. thaliana</i>	LL	3.91 \pm 0.82	209.9 \pm 8.31	1.44 \pm 0.52	0.999
	ML	4.63 \pm 0.24	542.0 \pm 49.2	1.21 \pm 0.04	0.999
	HL	5.90 \pm 1.04	1141.0 \pm 372.1	0.98 \pm 0.06	0.998
<i>N. palea</i>	LL	2.70 \pm 0.10	91.7 \pm 7.01	1.61 \pm 0.19	0.991
	ML	3.65 \pm 0.05	312.6 \pm 5.59	2.16 \pm 0.05	0.999
	HL	3.86 \pm 0.11	551.4 \pm 15.8	2.55 \pm 0.08	0.999

849

850 Table 3. Results of fitting of Eq. 2 to published NPQ vs. *E* curves. Composite: samples containing organisms with different XC types. LC: light curve protocol
851 (SS: steady state LL; RLCx: rapid light curve, x seconds light step; N-SLC: non-sequential light curve). Treatment: conditions applied before the measurement
852 of the light curves (PAR, temperature); growth conditions when more than one curve available for the same taxon (HL: high light; LL: low light; DTT: XC
853 inhibitor dithiothreitol; LL/HL: transfer from LL to HL; Dense: plants from dense forest stand; Edge: plants from forest edge; IL: intermittent light; CL:
854 continuous light; wt: wild type; *aba1*, *crr6*, *CRR6*, *lut1*, *lut2*, *lut2*, *npq1*, *npq2*, *npq4*, *npq5*, *vtc2*: mutants; LI-1, Sf-2, Col-0, Ws-2: *Arabidopsis* accessions;
855 Mixing: water column mixing). Parameter estimates \pm one standard error.
856

XC type	Taxon	LC	Treatment	Model parameters				Reference
				NPQ _m	E ₅₀	n	r ²	
VAZ	Magnoliophyta (flowering plants)	SS	wt 10 °C	2.07 ± 0.04	113.6 ± 6.0	1.11 ± 0.08	0.998	Havaux and Kloppstech (2001)
			wt 25 °C	2.98 ± 0.40	1118.0 ± 293.8	1.09 ± 0.11	0.996	
			<i>npq1</i> 10 °C	1.68 ± 0.25	636.5 ± 587.0	0.40 ± 0.06	0.998	
			<i>npq1</i> 25 °C	1.16 ± 0.22	867.1 ± 557.5	0.63 ± 0.10	0.995	
		SS	Li-1	3.76 ± 0.18	563.8 ± 40.6	1.83 ± 0.16	0.998	Jung and Niyogi (2009)
			Sf-2	4.12 ± 0.16	661.9 ± 33.7	2.03 ± 0.13	0.999	
			Col-0	3.29 ± 0.19	683.7 ± 55.0	1.84 ± 0.16	0.998	
			Ws-2	3.32 ± 0.19	599.8 ± 49.1	1.96 ± 0.21	0.997	
		SS	wt	2.06 ± 0.08	184.1 ± 13.4	2.21 ± 0.32	0.993	Lokstein et al. (2002)
			<i>lut1</i>	1.52 ± 0.06	195.5 ± 13.7	2.28 ± 0.33	0.994	
			<i>lut2</i>	1.76 ± 0.10	231.0 ± 22.2	1.72 ± 0.22	0.995	
			<i>aba1</i>	2.36 ± 0.19	251.1 ± 43.2	1.14 ± 0.13	0.996	
			<i>lut2aba1</i>	1.17 ± 0.07	214.7 ± 30.6	1.08 ± 0.10	0.997	

	SS	wt <i>vtc2</i>	3.22 ± 0.22 3.86 ± 0.22	822.2 ± 90.9 1606.8 ± 137.5	1.46 ± 0.15 1.32 ± 0.05	0.993 0.957	Müller-Moulé et al. (2002)
	SS	wt LL <i>vtc2</i> LL <i>vtc2npq4</i> LL <i>vtc2npq1</i> LL wt HL <i>vtc2</i> HL <i>vtc2npq4</i> HL <i>vtc2npq1</i> HL	2.99 ± 0.22 4.52 ± 1.08 2.03 ± 0.32 2.51 ± 1.41 4.55 ± 6.55 6.82 ± 2.35 1.82 ± 1.00 1.80 ± 1.84	993.6 ± 96.6 2492.1 ± 700.8 2556.4 ± 402.8 2597.0 ± 1777.5 4082.0 ± 5997.8 4133.0 ± 1851.3 3527.3 ± 2314.0 3502.8 ± 4087.1	1.95 ± 0.21 1.49 ± 0.15 1.73 ± 0.08 1.43 ± 0.26 1.47 ± 0.37 1.24 ± 0.10 1.39 ± 0.21 1.39 ± 0.32	0.998 0.999 0.991 0.998 0.998 0.999 0.996 0.996	Müller-Moulé et al. (2004)
	SS	wt <i>pgr5</i>	2.21 ± 0.10 1.77 ± 3.20	457.0 ± 26.5 1478.1 ± 5634.7	1.48 ± 0.16 0.81 ± 0.45	0.993 0.957	Munekage et al. (2002)
	SS	wt <i>crr6</i> <i>crr6CRR6</i>	2.25 ± 0.11 2.00 ± 0.07 2.01 ± 0.08	503.2 ± 39.4 397.7 ± 22.5 365.2 ± 24.2	1.44 ± 0.07 1.60 ± 0.08 1.65 ± 0.11	0.999 0.999 0.999	Munshi et al. (2006)
	SS	wt <i>npq1</i> <i>npq2</i>	4.47 ± 1.24 1.92 ± 0.81 3.23 ± 0.35	1894.4 ± 746.9 1877.4 ± 1686.5 1156.2 ± 207.4	1.34 ± 0.17 0.90 ± 0.18 1.32 ± 0.11	0.995 0.988 0.997	Niyogi et al. (1998)
<i>Begonia</i> × <i>erythrophylla</i>	SS	LL LL/HL HL	1.27 ± 0.04 1.77 ± 0.05 2.83 ± 0.17	388.3 ± 14.1 467.3 ± 15.8 618.3 ± 38.6	3.51 ± 0.36 2.95 ± 0.23 2.82 ± 0.32	0.992 0.997 0.995	Burritt and Mackenzie (2003)
<i>Hedera canariensis</i>	SS	LL LL+DTT HL HL+DTT	3.61 ± 0.04 1.14 ± 0.18 5.34 ± 0.33 1.48 ± 0.05	382.6 ± 6.3 656.2 ± 218.9 761.1 ± 59.6 437.0 ± 27.8	2.90 ± 0.13 1.09 ± 0.20 2.05 ± 0.24 2.20 ± 0.26	0.999 0.987 0.996 0.994	Bilger and Björkman (1990)
<i>Quercus petraea</i>	SS	Dense Edge	2.23 ± 0.03 5.55 ± 0.11	158.2 ± 6.35 423.2 ± 13.3	1.83 ± 0.13 2.04 ± 0.10	0.998 0.999	Rodríguez- Calcerrada et al. (2007)

		<i>Zostera marina</i>	SS	LL	0.87 ± 0.15	797.3 ± 302.0	1.01 ± 0.14	0.995	Ralph and Gademann (2005)
			SS	HL	3.12 ± 0.25	400.5 ± 65.3	1.30 ± 0.16	0.995	
			RLC5	HL	1.36 ± 0.06	276.9 ± 26.9	1.39 ± 0.17	0.997	
			RLC10	HL	2.34 ± 0.17	399.0 ± 60.7	1.02 ± 0.11	0.997	
			RLC40	HL	3.37 ± 0.15	360.7 ± 30.4	1.45 ± 0.12	0.998	
Bryophyta (mosses)		<i>Eurhynchium crassinervium</i>	SS		5.79 ± 0.58	237.9 ± 34.7	1.53 ± 0.12	0.998	Marschall and Proctor (2004)
		<i>Pogonatum urnigerum</i>			5.45 ± 0.48	584.7 ± 65.2	1.79 ± 0.15	0.998	
		<i>Polytrichum juniperinum</i>			5.90 ± 0.34	689.3 ± 34.3	2.68 ± 0.17	0.998	
		<i>Tortula (Syntrichia) ruralis</i>			12.23 ± 0.42	458.6 ± 16.5	2.61 ± 0.15	0.999	
		<i>Racomitrium aquaticum</i>			26.27 ± 3.23	830.8 ± 92.8	2.33 ± 0.18	0.999	
		<i>Trichocolea tomentella</i>			1.45 ± 0.06	68.7 ± 4.5	2.36 ± 0.33	0.992	
Chlorophyta (green algae)		<i>Chara corallina</i>	SS		1.67 ± 0.04	34.0 ± 0.5	6.80 ± 0.73	0.993	Krupenina and Bulychev (2007)
		<i>Chlamydomonas reinhardtii</i>	SS	wt	1.83 ± 1.46	1010.0 ± 1660.0	0.81 ± 0.15	0.992	Elrad et al. (2002)
				<i>npq5</i>	0.19 ± 0.01	56.2 ± 3.58	3.66 ± 0.74	0.986	
	<i>Picochlorum sp.</i>	SS		1.88 ± 0.29	410.2 ± 142.0	1.06 ± 0.20	0.975	Dimier et al. (2007a)	
Eustigmatophyceae		<i>Nannochloropsis oculata</i>	RLC10		1.01 ± 0.06	224.5 ± 31.7	1.01 ± 0.08	0.998	Cosgrove and Borowitzka (2006)
DD-DT	Bacillariophyceae (diatoms)	<i>Chaetoceros socialis</i>	SS		5.71 ± 0.08	149.4 ± 4.8	1.88 ± 0.10	0.999	Dimier et al. (2007b)
		<i>Skeletonema marinoi</i>			1.27 ± 0.03	233.0 ± 9.3	1.95 ± 0.13	0.998	
		<i>Thalassiosira rotula</i>			1.14 ± 0.18	198.9 ± 89.8	0.89 ± 0.23	0.977	
		<i>Fragilariopsis cylindrus</i>	SS	5 μmol m ⁻² s ⁻¹	1.00 ± 0.18	637.5 ± 266.1	1.08 ± 0.23	0.966	Kropuenske et al. (2009)
				65 μmol m ⁻² s ⁻¹	0.74 ± 0.08	1023.1 ± 233.6	1.05 ± 0.9	0.996	
				125 μmol m ⁻² s ⁻¹	0.59 ± 0.12	1024.5 ± 540.6	0.94 ± 0.13	0.986	
	<i>Phaeodactylum tricornutum</i>	SS		2.30 ± 0.10	367.6 ± 24.1	2.59 ± 0.45	0.984	Lavaud et al.	

	<i>Skeletonema costatum</i>			2.77 ± 0.75	2825.9 ± 1569.0	0.86 ± 0.08	0.998	(2007)
	<i>Thalassiosira oceanica</i>			0.88 ± 0.13	859.4 ± 282.2	0.95 ± 0.13	0.992	
	<i>Thalassiosira pseudonana</i>			1.05 ± 0.04	276.8 ± 18.4	1.96 ± 0.22	0.993	
	<i>Thalassiosira weissflogii</i>			1.58 ± 0.03	227.1 ± 8.3	2.70 ± 0.22	0.996	
	<i>Phaeodactylum tricornutum</i>	SS	IL	9.30 ± 0.10	288.5 ± 6.2	2.77 ± 0.16	0.999	Lavaud et al. (2002)
			CL	2.16 ± 0.08	334.4 ± 17.2	3.22 ± 0.71	0.980	
	<i>Navicula phyllepta</i>	RLC10		0.19 ± 0.06	1057.6 ± 731.3	1.01 ± 0.36	0.976	Perkins et al. (2006)
			RLC30	0.54 ± 0.07	972.2 ± 370.1	0.73 ± 0.11	0.997	
			RLC60	0.91 ± 54.5	450.0 ± 54.5	1.55 ± 0.28	0.990	
			SS	2.90 ± 0.57	1152.8 ± 463.0	1.10 ± 0.26	0.989	
	<i>Nitzschia palea</i>	SS	LL	3.48 ± 0.17	115.8 ± 12.2	1.38 ± 0.15	0.991	Cruz and Serôdio (2008)
			HL	5.62 ± 0.24	399.9 ± 27.1	1.58 ± 0.08	0.999	
Dinophyta (dinoflagellates)	<i>Symbiodinium</i> sp.	RLC5	25 °C	0.15 ± 0.01	143.3 ± 10.5	2.39 ± 0.32	0.996	Ralph et al. (2001)
			33 °C	0.53 ± 0.05	115.8 ± 22.5	1.21 ± 22.5	0.994	
			37 °C	1.20 ± 0.05	77.3 ± 6.7	1.18 ± 0.08	0.998	
			38 °C	0.37 ± 0.01	39.9 ± 2.2	1.66 ± 0.17	0.995	
Haptophyta	<i>Phaeocystis antarctica</i>	SS	$5 \mu\text{mol m}^{-2} \text{s}^{-1}$	0.27 ± 0.03	426.7 ± 49.7	1.93 ± 0.29	0.990	Kropuenske et al. (2009)
			$65 \mu\text{mol m}^{-2} \text{s}^{-1}$	1.49 ± 1.01	2953.0 ± 3309.0	1.07 ± 0.27	0.985	
			$125 \mu\text{mol m}^{-2} \text{s}^{-1}$	0.84 ± 0.13	755.1 ± 175.0	1.94 ± 0.56	0.986	
Composite	Microphytobenthos	RLC50		4.09 ± 0.12	349.2 ± 17.8	2.39 ± 0.26	0.994	Herlory et al. (2007)
			N-SLC	3.72 ± 0.13	546.2 ± 35.5	1.49 ± 0.10	0.996	
	Phytoplankton	SS	No mixing	5.07 ± 0.52	546.6 ± 85.5	1.42 ± 0.13	0.998	Kromkamp et al. (2008)
			Mixing	1.85 ± 0.09	446.4 ± 21.9	2.73 ± 0.24	0.997	

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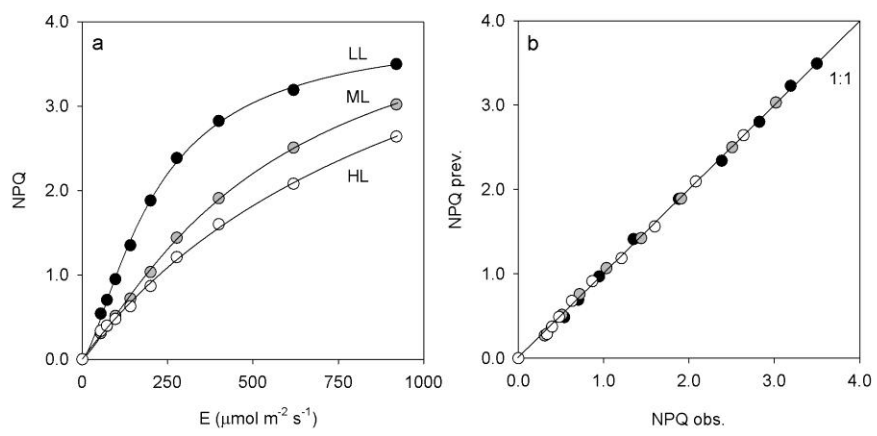
859 Table 4. Summary of the meta-analysis of the results of the fitting of the model to published
 860 NPQ vs. E curves (listed in Table 3). Mean values \pm one standard error.
 861

XC type	Model parameters		
	NPQ _m	E_{50}	n
VAZ	3.30 \pm 0.49	926.7 \pm 135.1	1.77 \pm 0.13
DD-DT	1.88 \pm 0.41	657.9 \pm 145.9	1.60 \pm 0.14
Mixed	3.68 \pm 0.67	472.1 \pm 47.3	2.01 \pm 0.33
All	2.89 \pm 0.35	824.3 \pm 99.2	1.73 \pm 0.09

862

863
864 Figure legends
865
866 Fig. 1. Fitting of Eq. 2 to light-response curves of NPQ measured in the plant *A. thaliana* grown
867 under low (LL), moderate (ML) and high light (HL). a. Light-response curves (data points),
868 fitted model (lines), and estimates of model parameters. b. Residuals of the model fitting.
869
870 Fig. 2. Fitting of Eq. 2 to light-response curves of NPQ measured in the diatom *N. palea* grown
871 under low (LL), moderate (ML) and high light (HL). a. Light-response curves (data points),
872 fitted model (lines), and estimates of model parameters. b. Residuals of the model fitting.
873
874 Fig. 3. Comparison between the light-response curves of NPQ and of ETR of *A. thaliana* grown
875 under low (LL), moderate (ML) and high light (HL). Estimates of the parameters of the model
876 of Eilers and Peeters (1988) fitted to the ETR vs. *E* curves are presented. Estimates of the
877 parameters of the curves fitted to the NPQ vs. *E* curves are presented in Table 2.
878
879 Fig. 4. Comparison between the light-response curves of NPQ and ETR of *N. palea* grown
880 under low (LL), moderate (ML) and high light (HL). Estimates of the parameters of the model
881 of Eilers and Peeters (1988) fitted to the ETR vs. *E* curves are presented. Estimates of the
882 parameters of the curves fitted to the NPQ vs. *E* curves are presented in Table 2.
883
884 Fig. 5. Relationship between the parameter E_{50} of Eq. 2 fitted to the NPQ vs. *E* curves and the
885 parameter E_k of the Eilers and Peeters (1988) model fitted to the ETR vs. *E* curves, for the plant
886 *A. thaliana* (circles) and the diatom *N. palea* (squares) grown under high (white), moderate
887 (gray) and low light (black).
888
889 Fig. 6. Comparison between the ratios E_{50}/E_k (a) and $\text{NPQ}(E_k)/\text{NPQ}_m$ (b) as calculated for the
890 published (excluding cases with impaired XC operation) and experimental (this study) light-

891 response curves of NPQ and ETR in VAZ and DD-DT types of XC. Bars represent one standard
892 error, boxes represent the lower (25%) and upper (75%) quartiles, and the thin and thick
893 horizontal lines inside the boxes represent the mean and the median, respectively.
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