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Functional Plant Biology

Supplementary Material

Validation of parameters and protocols derived from chlorophyll *a* fluorescence commonly utilised in marine ecophysiological studies

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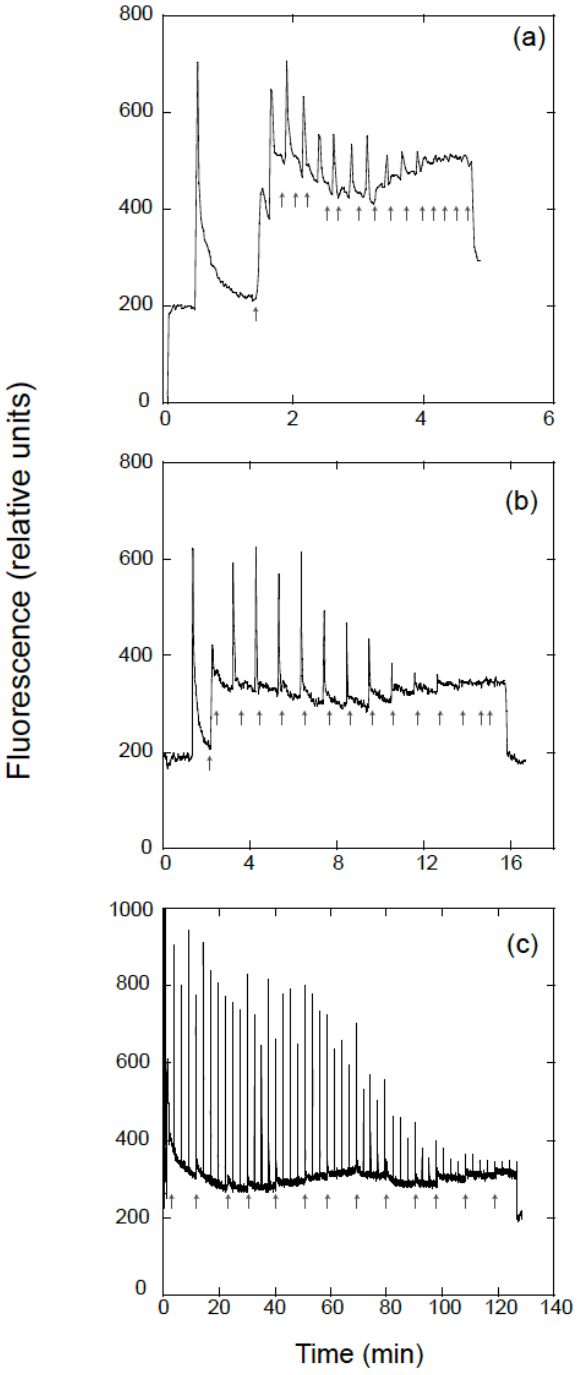

Figure S1. Variation of the chlorophyll *a* fluorescence kinetics of the leaves of the seagrass *Halodule wrigthii* while performing a complete light curve using 13 light treatments, using (a) and induction time of 10s; (b) an induction time of 1min; and (c) inductions times between 5-10 min that allowed achieving the photosynthetic steady-state at each light treatment. Arrows indicate a change in light treatment. Individual plots represent a single experiment of a set of 6 samples.

When performing a complete light curve using three different inductions times and 13-14 light treatments on the leaves of the seagrass *Halodule wrigthii*, we observed significant differences for the fluorescence signal of each curve, as illustrated in Figure S1. Two of the curves (Fig. S1a,b) were RLCs with induction times of 10 s and 1 min, whereas the third curve was a steady-state curve, SLC (Fig. S1c). The first RLC (10 s) only required 5 min to be completed, while the second curve (1 min) needed almost 16 min (Fig. S1a,b). Almost two hours were necessary to complete the 14 treatments of the SLC (Fig. S1c). These differences clearly explain the advantages of the RLC protocol. However, all of the $\Delta F_v/F_m'$ determinations were significantly lower than those measured with the SLC protocol, which showed much lower basal fluorescence (F_t; Fig. S1c).

The RLC that used the shortest induction times (10 s) showed the largest differences with the SLC determinations. A strong quenching of F_m' affected the capacity of the RLCs to measure $\Delta F_v/F_m'$ at the highest light treatments, as the fluorescence peak (F_m') was indistinguishable from the basal noise of the signal (F_t; Fig. S1a,b). This problem did not affect the SLC protocol, despite the same measuring light of the fluorometer was used (Fig. S1c).