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# Photosynthetic responses to $Cu^{2+}$ exposure are independent of light acclimation and uncoupled from growth inhibition in *Fucus serratus* (Phaeophyceae)

Hanne Dalsgaard Nielsen [*], Søren Laurentius Nielsen

*Department of Life Sciences and Chemistry, Roskilde University, P.O. Box 260, 4000 Roskilde, Denmark*

## Abstract

We have studied the effect of light acclimation on photosynthetic responses and growth during $Cu^{2+}$ exposure (0–0.84 μM) in the brown seaweed *Fucus serratus*. Measurements of chlorophyll fluorescence parameters showed that $Cu^{2+}$ exposure amplified ETR, reduced the chlorophyll content at the cellular level and that there was no effect of light adaptation on the $Cu^{2+}$ resistance of the algae. In contrast to the inhibitory effects of $Cu^{2+}$ on chlorophyll fluorescence, $O_2$ evolution and the total content of chlorophyll and carotenoid of the algae was unaffected by $Cu^{2+}$. We conclude that photoinhibition and perhaps pigment degradation in the meristoderm was compensated for by cells deeper in the thallus with the result that the overall photosynthetic fitness of the algae was maintained. The pronounced inhibitory effects of $Cu^{2+}$ on algae growth was not a consequence of photoinhibition and could be attributed to direct inhibitory effects on the growth process.

*Keywords:* Copper; Tolerance; Ecotoxicology; Macroalgae; Photosynthesis; Xanthophyll

## 1. Introduction

In response to human activity, toxic concentrations of $Cu^{2+}$ may eventually build up in certain estuarine and shallow coastal marine habitats near mining and industrial hot spots. In the temperate latitudes, the vegetation in such pollution vulnerable areas is often dominated by brown macroalgae (e.g. *Fucus serratus*) that naturally possess some resistance to $Cu^{2+}$. *F. serratus* which is indigenous to the North Atlantic is a macroalgae, that grows in the lower intertidal and subtidal zones. In addition to its importance as a primary producer, it provides shelter and substratum for a variety of epiphytic algae and marine animals.

Although $Cu^{2+}$ is essential for the metabolism of all eukaryotic organisms it is generally regarded as being one of the most toxic heavy metals that may target various metabolic processes. In particular, $Cu^{2+}$ is a potent inhibitor of photosynthesis in phytoplankton (Cid et al., 1995) and macroalgae (Küpper et al., 2002). The complex action of $Cu^{2+}$ in photosynthesis may primarily target the reaction centre of photosystem II (PSII) (Yruela et al., 1996) although damage to light harvesting chlorophyll a may also be involved (Küpper et al., 1996; Ciscato et al., 1997). The inhibitory effects of $Cu^{2+}$ on photosynthesis are similar to those caused by photoinhibition during which elevated light irradiances in excess of the photosynthetic requirements causes a reduction in the quantum yield of PSII (ΦPSII). In *F. serratus*, the high $Cu^{2+}$ tolerance of populations growing in polluted habitats may be related to photosynthetic adaptation (Nielsen et al., 2003a). However, detailed studies of the involvement of the photo-protective mechanisms in the natural resistance of brown algae to $Cu^{2+}$ are lacking.

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[*] Corresponding author. Tel.: +45 46 74 27 85.
*E-mail address:* hnielsen@ruc.dk (H.D. Nielsen).

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In the present study we aim to derive further insight into the mechanisms of $Cu^{2+}$ toxicity and tolerance in the photosynthesis of *F. serratus* with particular reference to light adaptation. The susceptibility of the photosynthetic apparatus of light and shade acclimated algae to long term (13 days) $Cu^{2+}$ exposure is assessed by studying the light response of the photosynthetic apparatus by measuring chlorophyll fluorescence parameters and oxygen evolution. The performance of the photosynthetic apparatus is compared to the composition of photosynthetic pigments of the experimental algae as measured by HPLC as well as the relative growth rate (RGR).

## 2. Materials and methods

Vegetative individuals of *F. serratus* were collected in March 2004 from a population near Hundested on the North coast of Sealand, Denmark by snorkeling and transported to the laboratory submerged in seawater at ambient temperature (below 15 °C) within $1\frac{1}{2}$ h. Apical frond tips (approximately 3 cm = fronds) were cut from the thallus and allowed to recover in filtered and UV treated seawater (FSW) at a temperature of 15 °C for two weeks during which FSW was changed every 2–3 days. During the recovery period fronds were acclimated to either 50 μmol $m^{-2}$ $s^{-1}$ photosynthetic active radiation (PAR), referred to as low light fronds, or μmol 500 μmol $m^{-2}$ $s^{-1}$ PAR, referred to as high light fronds, that was provided by mercury lamps on a 16h / 8h light/dark cycle. Subsequent to the recovery and light acclimation, while still kept at either 50 or 500 μmol $m^{-2}$ $s^{-1}$ PAR, fronds were transferred to individual beakers containing 150 ml of an artificial seawater medium (ASW) that was modified from the original recipe (Morel et al., 1979) and which allowed full control of the initial $Cu^{2+}$ concentration ([$Cu^{2+}$]) to which the algae were exposed. ASW was enriched with $CuSO_4 \cdot H_2O$ to yield total copper concentrations of 0 (control), 0.5 and 2 μM which was equivalent to [$Cu^{2+}$] in ASW of 0, 0.21 and 0.84 μM (Bond et al., 1999; Nielsen et al., 2003a). The ASW was changed every two days to compensate for ligand release by the algae (Gledhill et al., 1999). Effects on the photosynthetic performance of the algae was assessed by comparing the responses of chlorophyll fluorescence parameters to that of oxygen evolution as well as the daily relative growth rate after 5 and 13 days of exposure to $Cu^{2+}$. Similarly the content of chlorophylls and accessory pigments of the algae was determined. Six replicates of each light treatment were exposed to each of the three $Cu^{2+}$ treatments.

Chlorophyll fluorescence parameters were measured at 15 °C with a pulse-amplitude-modulated fluorescence monitoring system (IMAGING-PAM, Walz, Effeltrich, Germany) based on the principles of Schreiber et al. (1986) and carried out as described previously (Nielsen et al., 2003a) by application of the rapid light curve technique (White and Critchley, 1999). Fronds were placed at the water/air interface in a petri dish containing ASW. Focusing the transmission image of the frond ensured a fixed camera working distance, and digital images of chlorophyll fluorescence of fronds exposed to 18 intensities of actinic light increasing from 0 to 1210 μmol $m^{-2}$ $s^{-1}$ PAR were recorded during a 5 min time series. Numerical values of chlorophyll fluorescence parameters were extracted from the digital images using analytical software (IMAGING-WIN, Walz, Effeltrich, Germany). The electron transport rate of PSII (ETR) was calculated as:

$$\mathrm{ETR} = ((F_{m'} - F_t)/F_{m'}) \times 0.84 \times 0.5 \times \mathrm{PAR}(m^{-2}s^{-1})$$

(Maxwell and Johnson, 2000)

$F_{m'}$ and $F_t$ are the respective maximum and steady state fluorescence in the light. 0.84 is the approximately proportion of incident light that is absorbed by the algae of which a proportion of approximately 0.5 is transferred to PSII. The non-photochemical quenching (NPQ) of PSII was calculated as:

$$\mathrm{NPQ} = (F_m - F_{m'})/F_{m'}$$

and the maximum efficiency of PSII was calculated as:

$$F_v/F_m = (F_m - F_o)/F_m$$

(Maxwell and Johnson, 2000)

$F_v$ is the difference between $F_m$ and $F_o$ that are the respective maximum and minimum fluorescence of the dark adapted stage of PSII.

Continuous photosynthetic $O_2$ evolution of fronds was measured in a closed system fitted with a Clark-type oxygen electrode (Hansatech Instruments, Norfolk, UK). The current across the electrode was calibrated to oxygen-depleted and oxygen-saturated ASW. Individual, dark adapted (>8 h) fronds were submerged in 15 ml of ASW in the electrode chamber and oxygen evolution was measured at seven incident light intensities increasing from 0 to 800 μmol $m^{-2}$ $s^{-1}$ PAR upon reaching steady state photosynthesis after approximately 2–3 min. The initial slope ($\alpha$) of light response curves (which is proportional to $F_v/F_m$; Falkowski and Raven, 1997) in both ETR and $O_2$-evolution measurements was calculated by applying linear regression to the initial, light-limited part of the curve whereas values of the maximum electron transport rate as well as photosynthetic capacity ($ETR_{max}$, $P_{max}$) were calculated from the light-saturated plateau. Values of supra-saturating light intensities at which photoinhibition was initiated were also determined.

Daily relative growth rates (RGR) of the fronds were based on fresh weight (FW) measurements.

After 13 days of exposure to $Cu^{2+}$, the algae content of chlorophylls (a and c) as well as that of carotenoids (viola-, anthera-, zea- and fucoxanthin) was measured