Journal of Applied Phycology **9:** 255–259, 1997.

Inhibition of *Ectocarpus siliculosus* infestations with copper chloride in tank cultures of *Gracilaria gracilis*

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Received 23 March 1997; revised and accepted 16 June 1997

Key words: copper chloride, *Ectocarpus siliculosus*, epiphyte control, *Gracilaria gracilis*, *Haliotes midae*, tank culture

Abstract

This study investigated copper chloride as an inhibitor of infestations of *Ectocarpus siliculosus* in *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine and Farnham grown in outdoor tank cultures. Copper at concentrations of 400 and 800 μg L^{-1} Cu^{2+} inhibited *E. siliculosus* in one experiment. A reduction in the specific growth rates of *G. gracilis* was found for both concentrations of copper chloride in two experiments. *G. gracilis* recovered completely within three weeks in one experiment, while no recovery was found in the other. Three and 5 weeks after treatment with low and high concentrations of copper chloride respectively the specific growth rates of *G. gracilis* was higher than that of the control in one experiment. A feeding experiment was conducted to determine the effect of copper-treated *G. gracilis*, used as fodder, on the growth rates of *Haliotes midae* (abalone) of two size classes. An isolated occurrence of growth rate inhibition of *H. midae* could be demonstrated in both size classes 3 months after the start of the experiment.

Introduction

Epiphytism is a major problem in the seaweed mariculture industry (Kuschel & Buschmann, 1991; Fletcher, 1995). This problem is especially evident in tank culture systems (Lapointe & Ryther, 1978) due to unnatural growth conditions which increase the susceptibility of the host plant to fouling (Fletcher, 1995).

Various chemical methods of epiphyte control, including copper chloride treatments in pond cultures (Haglund & Pedersén, 1993), have been implemented with varying degrees of success. The use of copper-based paints to prevent epiphytic fouling in fish farming and on ships is a well known procedure (Evans, 1981; Lewis & Metaxas, 1991). Haglund and Pedersén (1993) used 100 μg L^{-1} Cu^{2+} to reduce *Enteromorpha intestinalis* infestations in pond cultures of *Gracilaria tenuistipitata* without severely affecting the cultures. They concluded that copper may be of great importance in controlling epiphytic green algae. No investigations have thus far been conducted on the use of copper in tank cultures of *Gracilaria* spp. to control epiphytes. Burdin and Bird (1994) showed that copper can accumulate in the thalli of *Gracilaria tikvahiae*, possibly as a result of binding to cell wall polysaccharides. *Gracilaria* spp., treated with copper and then used as abalone fodder, could cause unknown side effects in these animals and should be investigated.

This study evaluated the use of different copper treatments as an inhibitor of *E. siliculosus* in tank cultures of *G. gracilis*. The implications of the various treatments on *G. gracilis* specific growth rates were determined while abalone, exposed to treated thalli, was monitored for any detrimental effects to their growth rates.

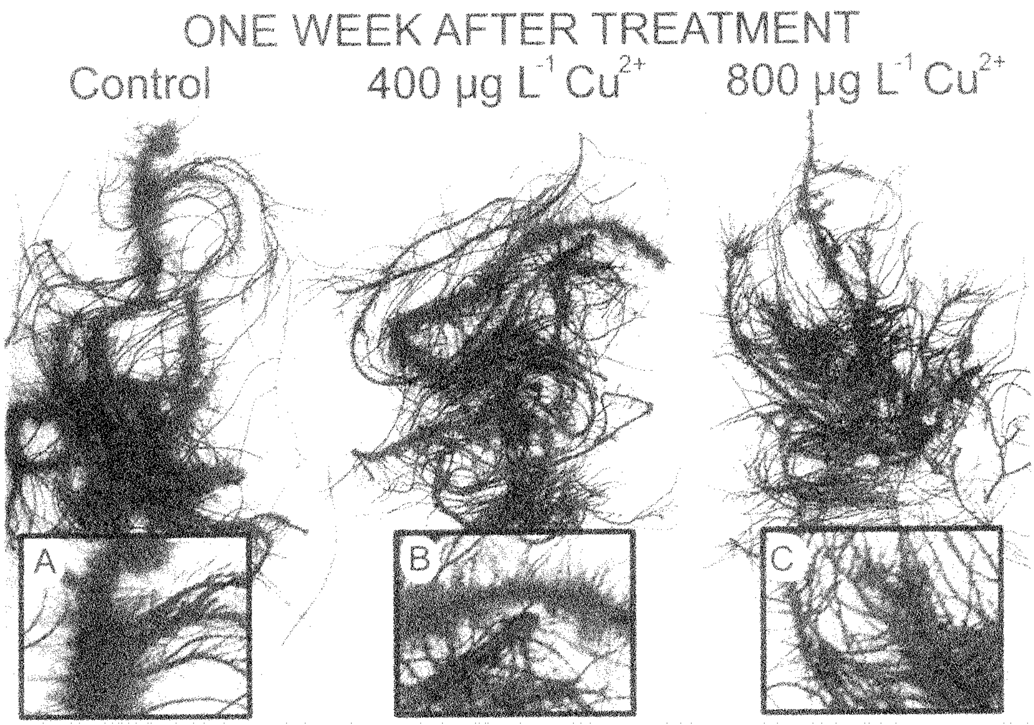

Figure 1. Influence of Cu^{2+} concentration on *E. siliculosus* infestation in tank cultures of *G. gracilis* one week after a single $CuCl_2$ treatment. Enlarged sections of thalli are shown in frames A, B and C at the bottom of the figure.

Materials and methods

The experiments were performed from June 1995 to January 1996 at Marine Growers Inc., an abalone farm near Port Elizabeth, South Africa. *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine and Farnham (Bird & Kain, 1995), previously known as *Gracilaria verrucosa* (Hudson) Papenfuss, is cultured at this site in large outdoor tanks and extensively used as abalone fodder. Vegetative thalli used as inocula in the experiments were collected from wild crops at Saldanha Bay, north of Cape Town, South Africa.

Culture conditions

Twelve outdoor canvas tanks (0.095 m^3, 0.223 m^2 surface area) supported in wooden frames were used for the cultures. Sea water was supplied by gravitational flow through 50 mm-ID PVC pipes from a large reservoir at a culture volume exchange rate of 48 volumes d^{-1}. Aeration was supplied continuously by an air blower through perforated 20 mm-ID PVC pipes. These pipes were located on the bottom edge of each tank resulting in a single circulation cell. Each tank was stocked with *G. gracilis* at an initial stocking density of 4 kg FW m^{-2} of tank area. Once weekly, the cultured alga was removed and weighed after vigorous shaking to remove excess water. The cultured alga was harvested to the initial stocking density, and after cleaning the tanks, the alga was returned. Once weekly at night, the water flow was stopped and the cultures fertilised for 10-h with 1200 μmol L^{-1} $(NH_4)_2SO_4$ and commercial phosphate fertiliser (N:P % ratio of 10:1).

Copper chloride treatments

Prior to the experiments, the cultures was grown for 3 months without inoculum renewal and became heavily infested with *E. siliculosus*. The cultures were treated with copper chloride in two separate experiments.

Experiment A
During September 1995 the cultures were divided in 3 groups of 4 replicate tanks each. The groups was treated with $CuCl_2$ for 48-h at Cu^{2+} concentrations of 0 μg L^{-1} (control), 400 μg L^{-1} and 800 μg L^{-1}.

These treatments represented concentrations respectively 4 and 8 times higher than those used by Haglund and Pedersén (1993) in their experiments. However, in those experiments the pond cultures were exposed continuously to copper, while in our experiments the treatments were administered in 48-h pulses. During the treatment period the water flow was terminated in all the groups.

For 5 weeks after the treatment the specific growth rate (SGR) of the 3 groups was determined once weekly as follows:

$$SGR(\%Fwd^{-1}) = \frac{100\ln Nt/No}{t}$$

where t is the time in days, N_o is the initial biomass and N_t is the biomass at time t. At the end of the experimental period the treated cultures were discarded and the control cultures distributed between the remaining tanks and cultivated until a stocking density of 4 kg FW m^{-2} was obtained once again in each tank.

Experiment B

During November 1995 the cultures were treated with $CuCl_2$ for 48-h at Cu^{2+} concentrations of 0 μg L^{-1} (control), 400 μg L^{-1} and 800 μg L^{-1}, respectively. One week later the same treatment was repeated. Although infestation with *E. siliculosus* was negligible, the experiment was conducted to determine the tolerance level of *G. gracilis* against repeated copper treatments. The SGR was determined for 4 weeks thereafter, as previously described in experiment A.

Haliotes midae *feeding experiment*

Tagged abalone (*H. midae*) of two size classes (1.7–2.2 cm and 3–3.5 cm respectively), were fed continuously for 5 months with 800 μg L^{-1} Cu^{2+}-treated *G. gracilis* thalli, while control animals fed on untreated thalli (25 abalone of each size class). The growth rates (cm $month^{-1}$) of the abalone were determined during the 5 month period.

Statistical analysis

The effect of the various $CuCl_2$ treatments and the 800 μg L^{-1} Cu^{2+}-treated thalli, on the growth rates of *G. gracilis* and *H. midae* respectively, were analysed statistically using a one-way ANOVA (P = 0.05).

Results

Effect on Ectocarpus siliculosus *infestation during experiment A*

The effect on *E. siliculosus* infestations one week after the Cu^{2+}-treatments are shown in Figure 1. A pronounced inhibitory effect on *E. siliculosus* could be seen in the *G. gracilis* cultures treated with 800 μg L^{-1} Cu^{2+}.

Effect on Gracilaria gracilis *specific growth rates*

Experiment A

One week after the treatment (Figure 2, week 1) both the 400 and 800 μg L^{-1} Cu^{2+} cultures showed a major inhibition in specific growth rate (SGR) of 3.5 and 2.64% FW d^{-1} respectively in relation to the control (5.05% FW d^{-1}). This SGR inhibition in both the 400 and 800 μg L^{-1} Cu^{2+} cultures was maintained until three weeks after the treatment (Figure 2, week 3) when a significant SGR stimulation was measured in the cultures treated with 400 μg L^{-1} Cu^{2+} (5.38% FW d^{-1}) by comparison with the control (4.84% FW d^{-1}). The cultures treated with 800 μg L^{-1} Cu^{2+} showed complete recovery 3 weeks after the treatment. Four weeks after the treatment (Figure 2, week 4) the SGR of the 400 μg L^{-1} Cu^{2+} cultures (7.3% FW d^{-1}) was higher than both that of the control (6.45% FW d^{-1}) and the 800 μg L^{-1} Cu^{2+} cultures (6.28% FW d^{-1}). Five weeks after the treatment (Figure 2, week 5) the SGR of both the 400 and 800 μg L^{-1} Cu^{2+} cultures (6.5 and 6.3% FW d^{-1} respectively) was higher than that of the control (5.06% FW d^{-1}).

Experiment B

A detrimental effect on the SGR of the 400 and 800 μg L^{-1} Cu^{2+} cultures could be seen throughout the 4 week post-treatment period (Figure 3, weeks 2–5). This inhibitory effect was especially evident in the 800 μg L^{-1} Cu^{2+} cultures which had a SGR of 2.25% FW d^{-1} compared to 7.65% FW d^{-1} of the control, 4 weeks after the second treatment (Figure 3, week 5).

Effect on Haliotes midae *growth rates*

The effect of Cu^{2+}-treated *G. gracilis*, used as abalone fodder over a 5 month period, on the growth rates of *H. midae* of two size classes was investigated (Figures 4A and B). The only significant effect on growth

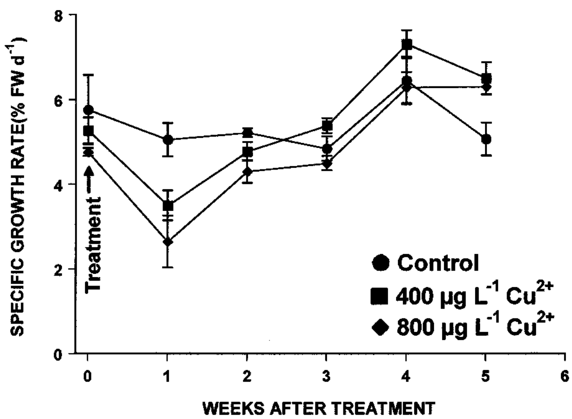

Figure 2. Influence of Cu^{2+} concentration on the mean specific growth rates of *G. gracilis* after a single 48-h $CuCl_2$ treatment. Bars represent the standard error.

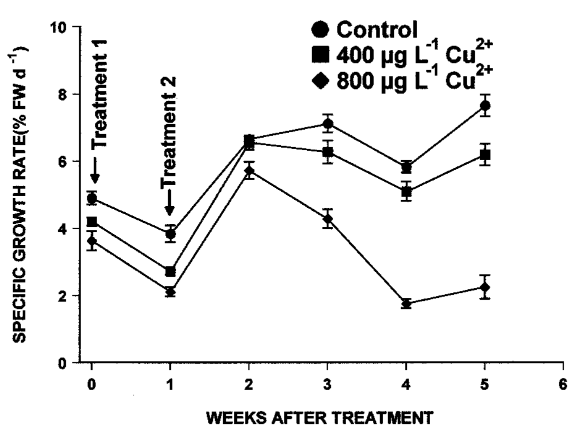

Figure 3. Influence of Cu^{2+} concentration on the mean specific growth rates of *G. gracilis* after two consecutive 48-h $CuCl_2$ treatments. Bars represent the standard error.

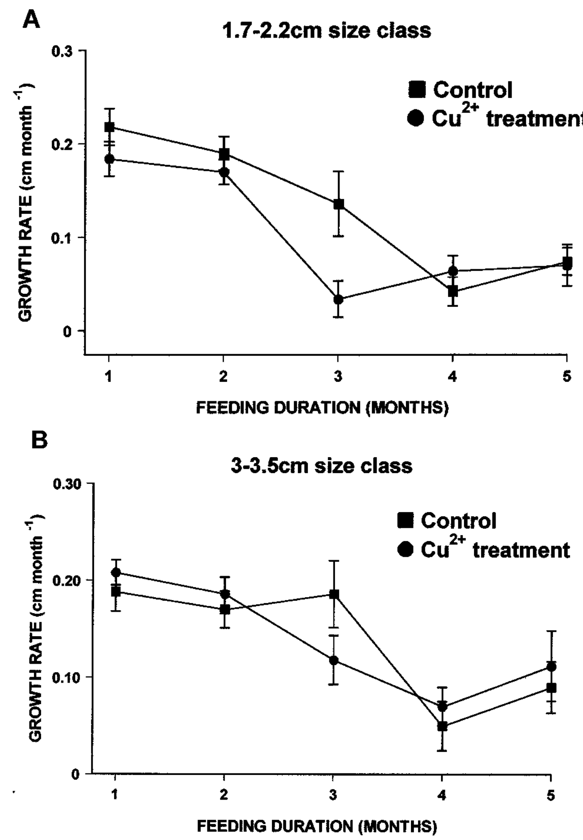

Figure 4. Influence of 800 μg L^{-1} Cu^{2+}-treated *G. gracilis* cultures, used as abalone fodder, on the mean growth rates of *H. midae* of two size classes during a 5 month period. A. 1.7–2.2 cm size class, B. 3–3.5 cm size class. Bars represent standard error.

rates was found 3 months after the initiation of the feeding experiment in both size classes (Figures 4A and B, month 3) where the treated abalone had lower growth rates than the respective controls. However, complete recovery in growth rates was observed 1 month later.

Discussion

Effect on the infestation of Ectocarpus siliculosus

The results indicate positive implications for cost-effective control of *E. siliculosus* infestations in tank cultures of *G. gracilis*. A pronounced inhibitory effect on *E. siliculosus* one week after a single 48-h treatment of $CuCl_2$ (experiment A) was observed. Haglund and Pedersén (1993) concluded that copper may be of great importance in controlling epiphytic green algae. Our results strongly suggests that copper treatments may also be effective in the inhibition of epiphytic brown algae such as *E. siliculosus*. Interspecific and intraspecific variation in copper tolerance is known to occur in *Ectocarpus* (Morris & Russell, 1974; Goodman et al., 1976) Further studies should be conducted to determine if the very low frequency of copper treatments, effective in controlling *E. siliculosus* at Marine Growers Inc. abalone farm, leads to copper-tolerant strain selection.

Effect on Gracilaria gracilis *specific growth rates*

Treatment of *G. gracilis* with $CuCl_2$ caused various degrees of SGR inhibition depending on the concentration and duration of the treatments. The results demonstrated that *G. gracilis* has a high degree of sensitivity to copper, with detrimental effects, when two consecutive treatments were administered. When a single copper treatment was administered (experiment A), the inhibition was only short-term. The stimulation of SGR in treated cultures in experiment A, could have positive implications for the mariculture industry. The stimulation of SGR could be the result of a decrease in competitive interactions between *E. siliculosus* and *G. gracilis*.

Effect on Haliotes midae *growth rates*

The results do not indicate any long-term inhibitory effects of copper-treated thalli on the growth rates of *H. midae*. Therefore, control of epiphytic infestations in *G. gracilis* cultures with $CuCl_2$ shows potential for use on abalone farms. The only inhibition of growth rates was found after three months of feeding in both size classes. This isolated occurrence of growth inhibition may indicate short-term copper-toxicity and tissue copper-analysis of treated *H. midae* should be conducted in future studies. In our experiments the abalone was fed continuously with copper-treated thalli for 5 months, in contrast to the very low frequency of copper treatments, needed to control *E. siliculosus* infestations. Small-scale implementation of $CuCl_2$ treatments at Marine Growers Inc. indicates promising results in controlling *E. siliculosus*. We propose that careful utilisation of copper treatments may be an effective method of epiphyte control in tank cultures of *G. gracilis* on abalone farms.

Acknowledgements

The provision of facilities and technical support by Mr Connie Muller and staff of Marine Growers Inc. is gratefully acknowledged.

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