Effect of copper on the scope for growth of clams () from a farming area in the Northern Adriatic Sea

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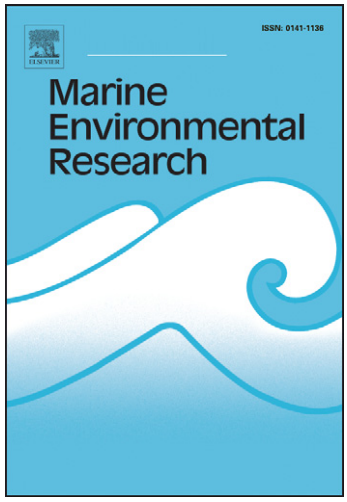

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Effect of copper on the scope for growth of clams (*Tapes philippinarum*) from a farming area in the Northern Adriatic Sea.

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Abstract

Copper is currently the most common biocide in antifouling paints. Levels of this metal were measured in the water, particulate matter and sediments from a shellfish farming area in the Sacca di Goro (Northern Adriatic Sea) over one year. With respect to the 1980s, copper environmental level increased two–fold. The release of copper from shellfish farmer boats was also estimated to be > 250 kg Cu y^{-1}. Clams *Tapes philippinarum* were collected in the same area and seasonally exposed to a sublethal (10 µgCu l^{-1}) concentration of copper. Physiological traits were significantly affected by copper exposure (scope for growth declined as a result of reduced clearance rate, increased oxygen consumption and a generally lower absorption efficiency). Results of this study are cause for concern for shellfish farming activities at least in Northern Adriatic, where shellfish farming is a monoculture of *T. philippinarum.* A strict interpretation of the precautionary principle might suggest that more rigorous regulatory action to control copper inputs in the field would be justified.

Keywords: Antifouling Paints; Copper; Scope for Growth; *Tapes philippinarum*; Adriatic Sea.

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Introduction

Copper is essential for biological life, since it is a cofactor of the prion protein and many metalloenzymes, such as cytochrome-c oxidase and copper-zinc superoxide dismutase (White and Rainbow, 1985; Geret et al., 2002). On the other hand, copper at high concentration is extremely toxic: because of its high affinity for thiol groups, copper is able to bind to cysteine with a consequent inactivation of the proteins themselves (Freedman et al., 1989). Copper pollution can arise from mining, brass manufacturing, electroplating and excessive use of copper-based agrochemicals. After the ban of the use of tributyltin as antifouling agent on small (< 25 m length) boats, copper has become the most common biocide in many antifouling paints (Yebra et al., 2004), and is the predominant toxicant among a number of other biocides (Okamura et al., 2002). Recent investigations have concluded that boat traffic may be a significant source of metal contamination, e.g. through the use of zinc anodes and copper-based antifouling paints (Matthiessen et al., 1999; Karlsson and Eklund, 2004; Schiff et al., 2004). Bivalves accumulate copper in direct proportion to their environmental levels (Roesijadi, 1980). Responses to copper exposure (80 – 500 µg Cu l^{-1}) have been shown to vary from a reduction in filtration rate (Manley, 1983) to profound effects on protein metabolism (Viarengo et al., 1981), and to reductions in calcium homeostasis (Gnassia-Barelli et al., 1995). At even lower concentration (10 µg Cu l^{-1}), activities of antioxidant enzymes are reduced (Isani et al., 2003), and growth performance is affected (Sobral and Widdows, 1997).

Tapes philippinarum is an exotic species that was deliberately introduced in the 1980s in the Sacca di Goro, a brackish lagoon in the Po River deltaic area (northern Adriatic Sea, Italy), for the relaunch of a mollusc fishery. Today, about half of the bottom of the Sacca di Goro is dedicated to clam culture-based fishery. There is an abundant literature on the ecological characteristics of the species, largely because of its commercial interest (*e.g.*

Goulletquer and Heral, 1997). However, interactions and energetics of *T. philippinarum* in the new habitat have only recently been addressed (Mistri, 2004; Sgro et al., 2005a; 2005b). *T. philippinarum* is both a deposit feeder (i.e. it filters resuspended sediment) and a facultative filter feeder, and is available from farming grounds all year round.

Copper, though currently receiving less attention than organotin, will probably face similar restrictive legislation in the future. To date, one developed country, Sweden, has banned all toxic metal antifouling coatings in the Swedish Baltic Sea area (Rittschof, 2001). The main objective of this study was to determine the effect of a sublethal concentration of copper on the energetics of *T. philippinarum*. Related goals were to determine whether, due to the ban of TBT in antifouling paints, copper contamination in clam farming areas exists, and to estimate the release of copper from shellfish farmers' boats.

Materials and Methods

Physiological measurements

Tapes philippinarum were collected from a farming ground (44°48'55.01''N, 12°18'19.44''E), and allowed to acclimate to laboratory conditions for one week prior to experiments. Clams were exposed to a sublethal copper concentration of 10 μg l^{-1}, obtained by diluting a stock solution of 1 g l^{-1} of $CuCl_2$. There were two copper exposure tanks and two control tanks with 30 clams per tank (in 10 l of aerated seawater). Clams were fed daily on a culture of T-*Isochrysis* (batch: TISO15-1307839; BlueBiotech® Germany). Water was renewed every two days. Tanks and glassware were acid-washed prior to use, and tanks rinsed thoroughly with seawater prior to addition of clams. Sampling times were at 0, 2, 5, 9, 14 and 20 days. At each time point, four randomly chosen clams from each tank (two controls and two treatments) were taken for physiological measurements. Measurement methods are fully described in Sgro et al. (2005a), and followed the procedures outlined by Widdows and Staff

(1997). Briefly, clearance rate was measured in plastic beakers (2 l) of filtered (0.45 µm) seawater, with two clams per beaker plus one control (without clams). Initial T-*Iso* concentration was ca. 25000 cells ml^{-1}. Each trial started when the clams started to filter again, i.e. when they extended their siphons. Samples were collected from each beaker every 20 min for the duration (2 h) of the experiment. Clearance rate was measured as the volume of water cleared of chlorophyll-a per unit time, and was related to the dry tissue weight of clams (oven drying of soft parts for 48 h at 80°C). Oxygen consumption was measured individually in four closed chambers (200 ml) plus one control, with a calibrated OxyGuard® Handy-γ oxygen electrode, at the start and after 2 h incubation period. Oxygen consumption was also corrected for the dry weight of clams, and converted to energy equivalents according to Gnaiger (1983): 1 µmol O_2 = 0.456 J. The absorption efficiency was established by the ratio method of Conover (1966), which measures the efficiency with which organic material is absorbed from the ingested food, as the ratio of the fractions of food and faeces lost on ashing (4 h at 450°C on pre-weighed Whatman® GF/C filters). Faeces were collected from the beakers at the end of each clearance measurement. The scope for growth provides an instantaneous measure of the energy status of the clam: the energy that clams have available for growth and reproduction was finally determined by the equation of Warren and Davis (1967) which is based on the difference between energy absorbed from food consumed and energy losses via respiration. In bivalves, clearance and respiration rates depend on the physiological status, food ration, and to a lesser extent, season and temperature (Bayne et al., 1976; Sobral and Widdows, 2000; Riisgård et al., 2003). The first set of experiments was carried out in November 2003, and then experiments were repeated in March, May and July 2004. Physico-chemical water parameters were: temperature 10° to 25°C, salinity 26 to 28 and pH 8.0 to 8.2. A total of 480 clams was used (31.5 mm mean shell length $\pm$ 0.6 SD).

Environmental measurements

Field surveys focused on sampling of water, sediment and suspended particulate matter (SPM), and covered nine monthly sampling periods from February to December 2004. One fixed station (44°48'55.27''N, 12°18'18.89''E; depth 1.5 m), close to the farming ground, was chosen. At each sampling date, 50 l of seawater were collected and immediately vacuum-filtered onto Millipore® HA 0.45 μm membranes. Two litres of filtered water were retained and acidified with suprapur HNO_3 to 1-2 pH (2 ml l^{-1}). Membranes with SPM were transferred in weighed bottles and sealed. Sediment samples were collected with a corer; the first 2 cm of three replicate cores (i.d. 5 cm) were retained. All samples were ice-chilled in a cooler on the boat, and received at the laboratory within 2 h after collection. Water samples were analyzed through DPASV (Differential Pulse Anodic Stripping Voltammetry; Wang, 1994) with an AMEL433 instrument. This technique is particularly useful when analyzing seawater. Membranes with SPM were oven dried (100°C) to constant weight, treated with suprapur HNO_3, and mineralized in a microwave oven (Milestone ETHOS 900: 350 w: 15 min, 400 w: 10 min). After dilution with Milli-Q water, copper analyses were carried out through plasma spectrometry (Perkin-Elmer Optima 3100 XL). Sediment samples were treated and analyzed like SPM samples. Adequate quality assurance/quality control measures were followed in all aspects of the study, and intercalibration exercises were carried out within the TAQC-WFD (ISO/IEC 17025). The analytical procedures were checked using certified reference material (2704 of National Bureau of Standards) and allowed agreement with the certified values higher than 95%.

Estimation of copper inputs from shellfish farmer fleet

The estimate of copper inputs from boats was conducted calculating the wetted hull area (WHA) of boats, as WHA = L x (B + D), where L is the length, B the beam, and D the draft. Then, the leaching rate of copper from antifouling paints was calculated considering the

input for all boats (IB) per day, as IB = Number of boats x WHA x release rate of copper. The release rate of copper was drawn from literature (6.24 μg cm^{-2} d^{-1}; Matthiessen et al., 1999).

Results

No mortality occurred during the physiological experiments. Clearance rates were always lower in the copper-exposed clams (Fig. 1). Two-way ANOVAs (Table 1) showed a significant (all $p < 0.001$) initial decline in clearance rates of exposed clams, which tended to stabilize between the fifth and the eleventh day of exposure, depending on the period of experimentation. Clearance rates also differed with time of exposure (all $p < 0.001$). Oxygen consumption was always higher in exposed clams (Fig. 2; all $p < 0.001$ except November, $p < 0.05$). Absorption efficiency followed a more complicated pattern (Fig. 3). In two out of four experiments (November and March), a compensation seems evident in the earlier period of exposure, since less food was ingested (lower clearance rates) but it was absorbed more efficiently (higher absorption efficiency). However, all experiments showed a consistent decline in absorption efficiency after 9 d exposure. The results presented in Figures 1, 2 and 3 shows that there was generally a consistent physiological response to Cu in all four seasonal experiments. Scope for growth was calculated for each measurement period, and was always higher for control clams, independently from different environmental conditions and physiological status of the clams (Fig. 4). In March and July, negative values of the scope for growth indicate that Cu exposed clams had to use their body reserves to meet metabolic requirements.

Remarkable variations of copper values in the field were found during the study period. Table 2 summarizes copper concentration in water, SPM, and sediments. Briefly, water concentrations ranged between 2 and 7.5 μg Cu l^{-1}; in the SPM between 70 and 287 μg Cu g^{-1}, while in the sediment variations were between 7.5 and 53.5 μg Cu g^{-1}.

At Goro, shellfish farmers' boats are quite standard in size, and parameters for the calculation of WHA were: L = 5 m, B = 1.5 m, and B = 0.5 m. The fleet consists of about 1200 boats (IREPA, 2003). Thus, the leaching rate of copper from antifouling paints was estimated at 0.75 kg Cu d^{-1}, equalling 273 kg Cu yr^{-1}. However, this copper input must be considered a very conservative estimate, since it assumes one antifouling treatment per year (most shellfish farmers treat their boats twice a year), and does not consider the recreational fleet (about 200 yachts of various sizes) which is moored all year round in the touristic harbour of Goro.

Discussion

After their ban for the use on small boats and aquaculture, triorganotin antifoulants have been superseded by antifouling products based mainly on copper metal oxides. Although the deleterious effects of excess copper on aquatic biota are known, very few data are available regarding the potential increase in copper concentration following an increase in popularity of such products amongst small boat users. Pre-TBT-ban copper concentration in the water of the Sacca di Goro (1987-1988) was between 0.2 and 1.6 µg Cu l^{-1} (Fagioli et al., 1991). It seems evident that in the last 15 y, the concentration of copper in the water has greatly increased. Of course we can not state whether this is due only to the use of copper-based antifoulants, or to a generalized increase of contamination of the environment (e.g. through the discharge of continental water from the catchment basin of the Po di Volano river). Helland and Bakke (2002), however, reported that during a period of 90% reduction in the industrial input of copper in a Norwegian estuary, the very high concentrations of copper found were caused by leaching from antifouling paints. We estimated very conservatively a leaching rate of copper from antifouling paints of about 270 kg Cu yr^{-1}. This amount is comparable to figures reported in many major marinas and yacht moorings in U.K.

(Matthiessen et al., 1999), which are cause for concern. The sampling site chosen in the Sacca di Goro, being close to the shellfish farming ground, should be considered a "clean" site. Unfortunately, there are no data about pre-industrial levels of copper in the Sacca. However, from the 1980s, an increased concentration of copper in the water (up to 7 μg Cu l^{-1}), SPM (up to 250 μg Cu g^{-1}) and sediment (up to 50 μg Cu g^{-1}) is evident. Moreover, sedimentary copper concentrations were often above the threshold effect level (TEL: 18.7 μg Cu g^{-1}) and effect range Low (ERL: 34 μg Cu g^{-1}) of the Florida Department of Environmental Protection (FDEP; MacDonald, 1994) and the National Oceanic and Atmospheric Administration (NOAA; Long et al., 1995) sediment quality guidelines respectively. Copper is known to preferentially adsorb to organic matter, and such an adsorption might reduce the reactivity and hence the toxicity of copper (Gardner and Ravenscroft, 1991). Clams, however, can accumulate contaminants directly from seawater, from sediments and from contaminated food (Coelho et al., 2002a; 2002b; Coughlan et al., 2002). Such accumulation can result in the production of initiators of cytotoxic injury with degeneration of lysosomal function (Kurelec, 1993), decreased scope for growth, as shown by the present study, faster aging, and finally, decreased reproduction which reduces the fecundity of the population (Coughlan et al., 2002).

Most bivalves can reduce the toxic effects of metals by producing metal-binding proteins (Roesijadi, 1980). However, the increased energy expenditure recorded in the Cu exposed clams was probably due in part to the increased cost of metal-binding protein synthesis. When combined with the reduction in food energy consumption, due to the toxic effects of copper on the ciliary feeding activity, this resulted in very low and even negative values of scope for growth in 10 μg Cu l^{-1} exposed clams. Even at the end of the 20 d exposure period, when physiological responses seemed to recover slightly, the overall energetics of the clams was still greatly affected. This suggests that any detoxification mechanism allowed only partial recovery or only served to reduce the potential toxic effects

of Cu exposure. In fact, at the end of the 20 d long exposure, clearance rates were, on average, 54% of control rates, respiration was high at about 120% of control, and scope for growth was reduced to about 15% of the initial value. These figures are in accordance with Sobral and Widdows (1997), who studied the effect of copper on the clam *Ruditapes decussatus*. It has been suggested (Hawkins et al., 1989) that a positive relationship may exist between the growth rate of individual bivalves and heterozygosity measured at polymorphic enzyme loci. In the blue mussel (*Mytilus edulis*), exposure to copper in the laboratory revealed genotype-dependent mortality, with those individuals expressing a higher degree of heterozygosity surviving longest (Hawkins et al., 1989). The hypothesis that heterozygosity is associated with improved survivorship in contaminated conditions is also supported by Nevo et al. (1986), who reported that for marine molluscs, broad-niche and highly heterozygous species display significantly higher survivorship than narrow-niche, genetically poor, congeneric species, after exposure to inorganic pollutants. Unfortunately, the level of heterozygosity of Adriatic *Tapes philippinarum* populations is unknown. However, considering that all Adriatic populations, from Venice to Ravenna, derive just from three successive introductions (1984, 1985 and 1986) of only about 20 kg of seed imported from the same British hatchery (Pellizzato, 1990), it seems reasonable to suspect that the level of heterozygosity should not be particularly high. The increasing environmental levels of copper, as well as other toxic environmental contaminants, poses a threat to populations with reduced heterozygosity and how it will affect the species' fitness and distribution.

In summary, the data presented in this study give sufficient concern about possible environmental damage to further justify restrictive legislation for copper use in antifouling coating. A strict interpretation of the precautionary principle might suggest that more rigorous regulatory action to control copper inputs in the field would be justified.

Acknowledgemets

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Figure Legends

Figure 1. Mean clearance rates (CR) by clams (*Tapes philippinarum*) in response to Cu exposure (10 µg Cu l^{-1}) and control conditions (mean ± SD).

Figure 2. Mean rates of oxygen consumption by clams (*Tapes philippinarum*) in response to Cu exposure (10 µg Cu l^{-1}) and control conditions (mean ± SD).

Figure 3. Mean absorption efficiency (AE) by clams (*Tapes philippinarum*) in response to Cu exposure (10 µg Cu l^{-1}) and control conditions (mean ± SD).

Figure 4. Scope for growth for control and exposed clams (*Tapes philippinarum*) at each measurement period.

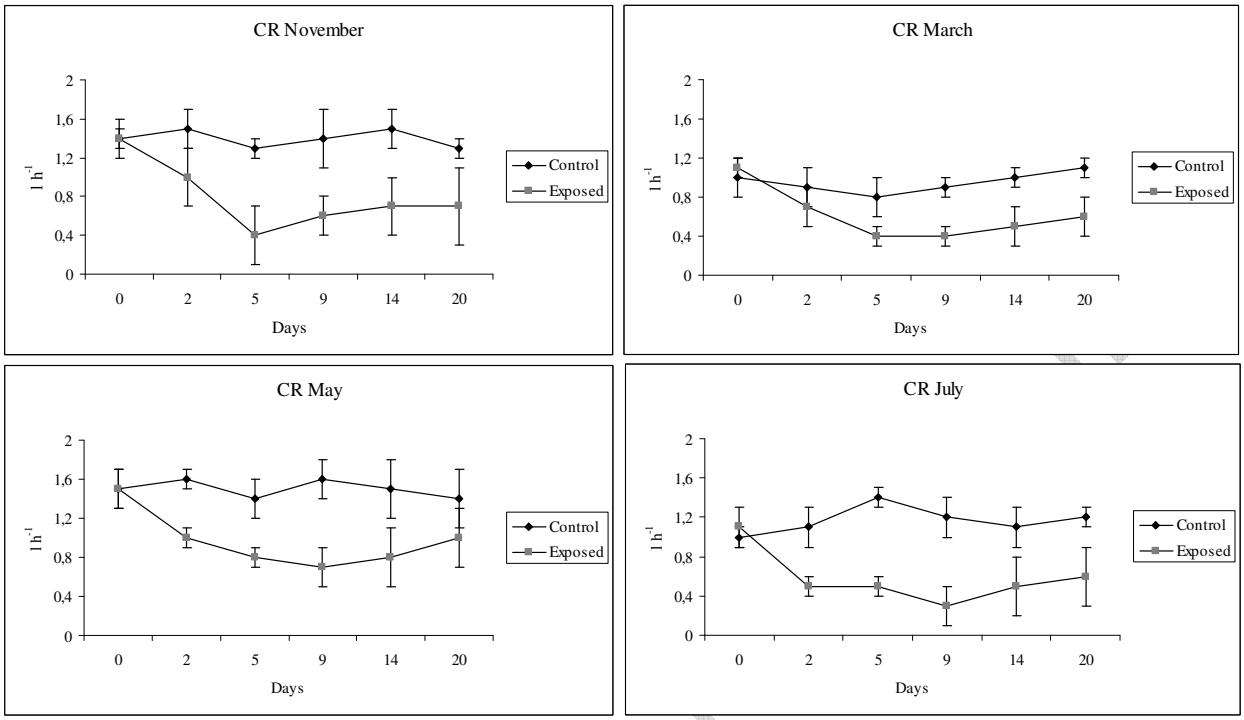

Figure 1

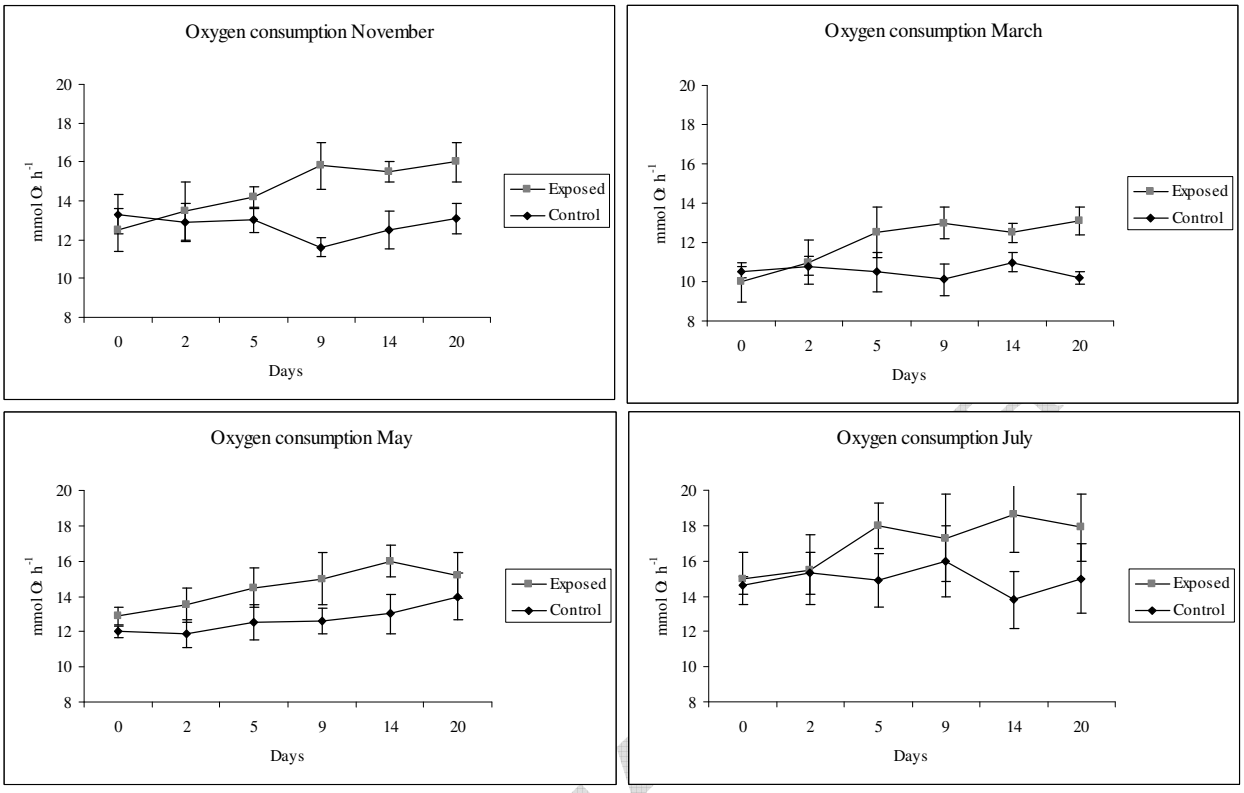

Figure 2

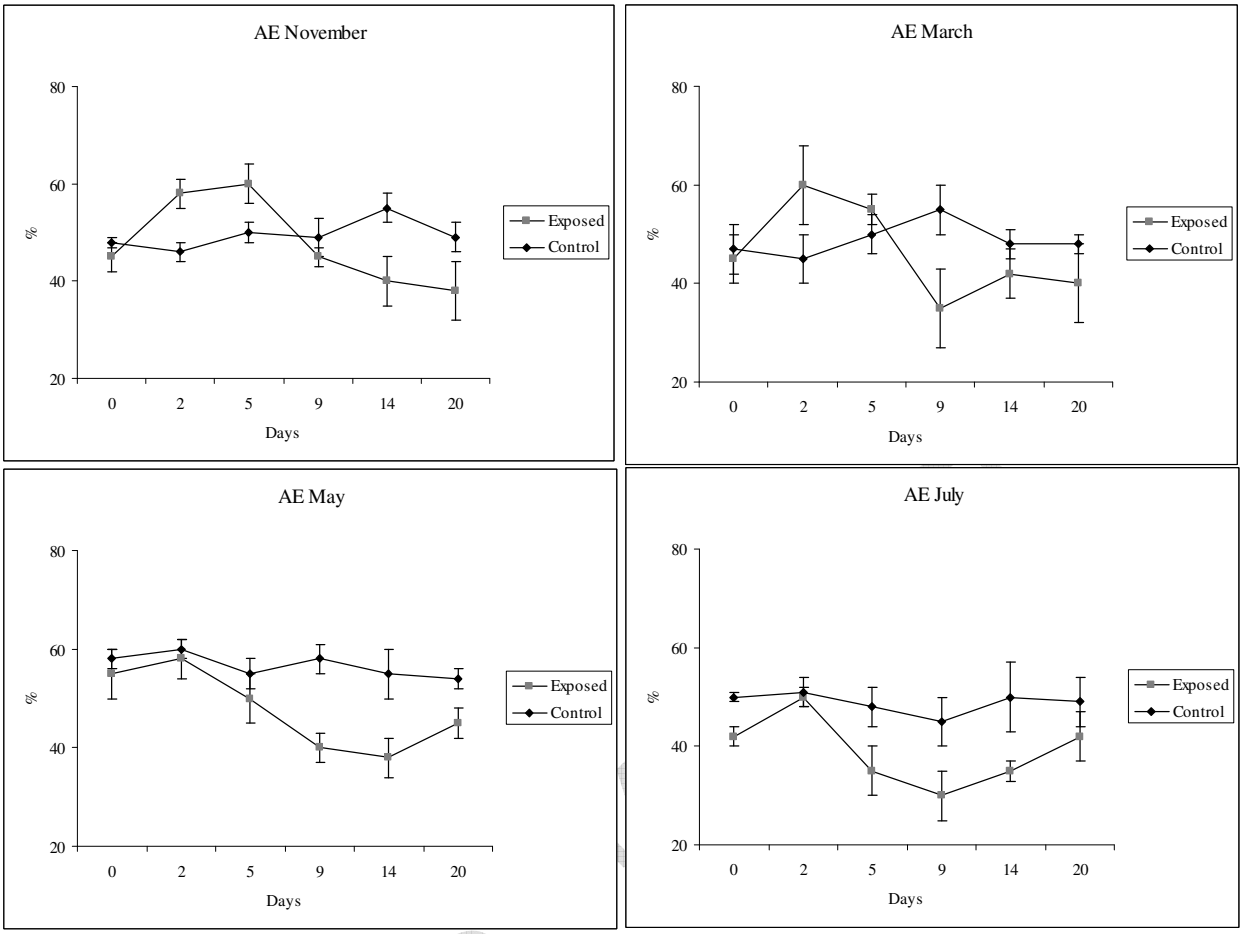

Figure 3

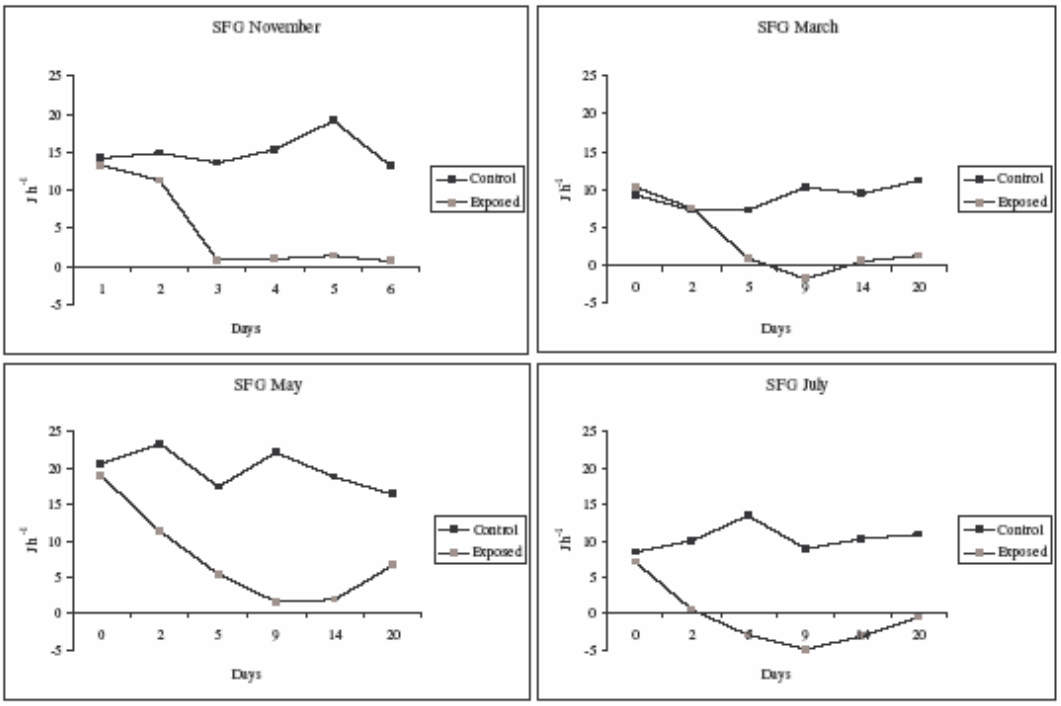

Fig 4

Table 1
Results of ANOVA (F values) for physiological measurements.
Significancy: ***: p<0.001; **: p< 0.01; *: p<0.05.

	Nov	Mar	May	Jul
Clearance rates				
Time	7.9***	8.8***	10.5***	2.4
Treatment	189.4***	106.7***	204.1***	110.2***
T x T	7.9***	8.6***	11.5***	8.1***
Oxygen consumption				
Time	1.6	1.6	8.1***	9.1***
Treatment	5.3*	60.1***	55.8***	99.2***
T x T	1.1	4.9**	0.8	8.6***
Absorption efficiency				
Time	7.1**	6.8***	9.8***	10.4***
Treatment	4.3*	8.3**	65.6***	88.4***
T x T	10.9***	12.2***	2.7*	3.3*

Table 2
Values of copper concentration (mean ± standard deviation) at the study site.

	Water ($\mu g\ Cu\ l^{-1}$)	SPM ($\mu g\ Cu\ g^{-1}$)	Sediment ($\mu g\ Cu\ g^{-1}$)
February	3.3±0.2	108.1±4.0	16.4±1.5
March	3.0±0.2	187.7±4.7	46.3±5.5
April	2.0±0.1	101.0±3.5	21.9±4.0
May	3.2±0.2	100.7±4.5	15.0±3.2
June	7.3±0.2	74.3±2.1	7.5±2.0
July	3.5±0.1	70.0±2.5	8.6±2.2
August	ND	ND	ND
September	ND	ND	ND
October	3.0±0.3	100.5±4.0	18.0±4.6
November	2.5±0.2	201.6±5.8	26.3±3.7
December	4.7±0.2	287.3±6.0	53.5±10.1