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Lethality of Copper Sulfate and Copper-Treated Nets to Juvenile Haddock, *Melanogrammus aeglefinus* L.

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Copper is the active ingredient in formulations of anti-fouling paints used on nets in finfish aquaculture. Antifoulants are employed to reduce the build up of biota on nets, thus allowing free flow of water and reducing the need to (and cost of) frequent net changes (Debourg et al.1993). While copper is essential for the normal growth and metabolism of all living organisms, abnormally low levels can result in nutritional deficiencies and excessive concentrations may be lethal (Eisler 1997). Sublethal effects on aquatic organisms have been demonstrated over concentration ranges from <100 □g/L to over 1000 µg/L (Hodson et al. 1979). The lethality of copper is well documented for many freshwater species of fish, however, very little work has been conducted with marine species (see for example, Hodson et al. 1979, Eisler 1997).

Recently, a lab experiment involving juvenile haddock (*Melanogrammus aeglefinus*), a potential new species for marine aquaculture, was abandoned due to a large loss of experimental animals. The suspected cause of death was copper leaching from a net freshly treated with copper-based antifouling paint. Although only observed in the lab, this mass mortality of fish raised concerns regarding the sensitivity of this species and potential effects of copper from antifoulants on non-target organisms – particularly fish held within the cage.

In order to begin to understand the consequences of copper usage in aquaculture, it is important to first define the lethal limits of copper to species of fish likely to be exposed to copper-based antifoulants during normal aquaculture practices. We herein report the results of lethality tests conducted with juvenile haddock exposed to copper ($CuSO_4$) and to freshly treated nets.

MATERIALS AND METHODS

Samples of treated net were analyzed for elemental copper by Atomic Absorption Spectrophotometry after acid digestion. Samples of netting were also analyzed for the presence and concentration of organotin compounds by gas chromatography – flame photometric detection (GC/FPD) after extraction in 0.1% tropolene/toluene.

Juvenile haddock were collected from the haddock aquaculture program at the St.

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Andrews Biological Station. They were held at 10°C in 2 m diameter tanks with free flowing water (salinity ~30 parts per thousand) until 12 hours prior to testing at which time they were transferred to treatment tanks. Netting (230g) (freshly treated with a copper-based (Cu_2O) antifouling paint) was used to line a glass aquarium (25.7 x 49.6 x 22.2 cm) filled with seawater (28.3 L). Five juveniles (fork length =14.3 (SD=1.3cm); weight = 31.7 (SD=7.5) g) were placed in the tank. The aquarium was kept in a water bath to maintain the seawater temperature at 9° C. The water was aerated and the level of dissolved oxygen remained >80% in each of the two static exposures. A control aquarium was lined with the same amount of untreated netting and haddock from the same stock of fish were held in this chamber.

Exposures were conducted under static conditions and the LT50 was calculated as the geometric mean of the longest length of exposure with less than 50% mortality and the shortest length of exposure where more than 50% of the haddock died.

Juvenile haddock (fork length = 16.2 (SD=1.5)cm; weight = 46.5 (SD=14.6) g) were exposed to copper sulfate in 200 L glass aquaria filled to 150 L (n=7 fish per aquarium). The aquaria were held in a 10°C water bath and water was aerated throughout the 48-hr static exposures, maintaining oxygen concentrations at >90% saturation. Haddock were exposed to five concentrations of copper sulfate ranging from 220 μg/L to 2000 μg/L (as copper). In addition, one group of fish was held in untreated seawater. The haddock were not fed during the exposures.

Water (5 mL) was sampled from the net-lined tanks at 0, 3, 5, 12, and 24 hr and at 0, 3, 6, 12, 24, and 48 hr from the $CuSO_4$ exposure. Water was analyzed for the presence of copper according the method of Engström et al. (1998). Briefly, water samples were mixed 1:1with a solution of 4-(2-pyridylazo) resorcinol (PAR), allowed to stand for 15 minutes and analyzed spectrophotometrically (λ = 490 nm). Copper was quantified by comparing samples from lethality tests to known standards of $CuSO_4$.

The loss of copper with time from the $CuSO_4$ exposure was described as a linear decrease and the values of intercept and slope were used to calculate the average measured copper concentration in each aquarium according to Zitko et al. (1977). The loss of copper with time was assumed to be the same for each concentration tested.

The LC50 was calculated according to Stephan (1977). The LC50 and confidence intervals were calculated by probit analysis when lethality data from an individual test included at least two concentrations where more than 0% yet less than 100 % of the exposed fish died. If the data did not meet this criteria, Spearman-Karber analysis was used.

RESULTS AND DISCUSSION

The average concentration of copper present on the nets to which haddock were exposed was 95 mg/g (CL Chou *personal communication* 2001). The analyst commented on the heterogeneity of the concentration from various parts of the treated net. The concentration of tri-n-butyl tin in the netting was approximately 0.27 µg/g (S. Batchelor *personal communication* 2000), a small fraction of the quantity of copper present. This concentration is also significantly lower than that found on tin-treated nets and unlikely to result in the release of sufficient TBT to kill finfish. The 96 hr LC50 for tributyl tin oxide in marine and estuarine finfish ranges from 2µg/L for larval sole (*Solea solea*) to 36 µg/L for adults of the same species (Hall Jr. and Pinkney 1985)

The copper concentration in water from net-lined aquaria rose to approximately 1800 µg/L after the first 5 hours. In each test the calculated LT50 was less than 6 hr (4.5 and 5.7 hr) indicating an acute response to the rapidly rising copper concentration.

Table 1 shows the calculated values for the lethality (48 hr LC50) of copper sulphate to juvenile haddock. The average LC50 over the four exposures was 412.5 µg/L with a standard deviation of 270. There is a four-fold difference in LC50 values indicating a considerable variability in the response. A fact that is confirmed by the observation that the 95% confidence interval for the overall mean includes 0. Figure 1 is an example of one of the lethality curves observed during work with juvenile haddock and copper sulphate.

Table 1. Calculated lethality (48hr LC50) for copper and juvenile haddock. Based on measured copper concentrations.

Test #	48 hr LC50 (µg/L)	95% confidence interval
1	290	210-410
2	170	150-190
3	400	300-520
4	790	660-960
mean	412.5	0-830

Lethality data are rare for marine finfish species and copper. Eisler (1997) reports an estimated LC50 of 448 µg/L for juvenile flounder (*Paralichthys spp.*) exposed to copper for 14 days. The 96 hr LC50 for winter flounder (*Pleuronectes americanus*) embryos is reported to be 126 µg/L, similar to the reported 96 hr LC50 for larval Atlantic silverside (*Menidia menidia* L.) of 136 µg/L (Eisler 1997). Our estimate of copper lethality to juvenile haddock (48 hr LC50 = 412.5 µg/L) is consistent with these values.

The risk of Copper-based antifouling paints posing a significant hazard in the marine environment is dependent on a number of factors. Copper-based paints are used in the aquaculture industry as antifoulants for treating nets used in cage structures. The authors contacted two companies actively involved in treating nets with copper-based paints in southwest New Brunswick. Two products,

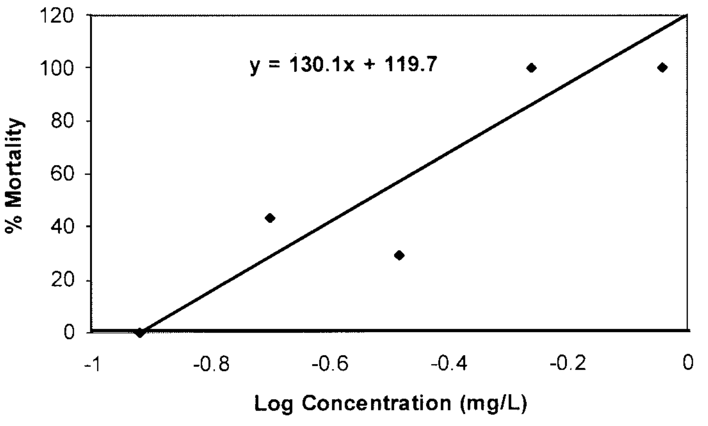

Figure 1. Representative lethality curve for copper and juvenile haddock.The average concentrations are derived from measured values.

Aquashield (26.5% Cu_2O, 23.5% elemental copper) and Flexgard (16.4 % Cu_2O, 14.5 % elemental copper) are in use. The methodology of treating nets is not very precise. Nets are either pulled through, or dipped in, a paint bath. The treatment is expected to increase the overall weight of the net by 30-35%, therefore adding approximately 8.2 or 5.1 g (depending on product) of elemental copper for every 100 g of treated net. In practice, the dipping technique may result in an increase in weight ranging from 5 to 65 %. This could result in a corresponding range of copper concentrations of 1.2 to 15.3 g per 100 g of net, again depending on product. The measured concentration of copper reported here falls in the predicted range.

The fate of copper is difficult to assess. In the lab, concentrations of up to 3 μg/L were observed in 32 L of water in which ~200g of treated net was soaked. This equals ~94 mg of copper, well under the minimum quantity of copper theoretically available (1.2g (copper)/100g (net)) using the paint with the lowest copper concentration and assuming the lowest incorporation of elemental copper. Work by Burridge et al. (1999) has shown elevated levels of copper in sediments near aquaculture sites relative to sites removed from aquaculture activities. The source of copper is unknown although it is likely that antifouling paints contribute to the load. Assessment of field effects and potential risks are complicated by the fact that individual finfish grow-out sites may have very different characteristics. Hydrographic conditions dictate not only how much copper may be released but also where it goes. Sites where low current may lead to an increase in the level of fouling on net pens may also be typified by slower release of copper from the treated net and longer exposure of the enclosed fish. In contrast, higher energy sites may have a larger release of copper from nets resulting in an increased copper concentration in the water. The elevated concentration may, in turn, be short-lived due to water movement.

It is suggested that wave action and agitation cause the net to lose its antifouling capacity quickly (B. Hill *personal communication* 2001). In addition, anecdotal information from farmers of Atlantic salmon suggest there is some loss of fish when new nets are put in place (B. Robertson *personal communication* 2001). These losses have been attributed to various causes, none of which include the possibility of elevated copper concentrations playing a role. The 96hr LC50 of copper to Atlantic salmon in freshwater ranges between 32 and 125 μg/L and is dependent on water hardness (Sprague et al. 1965). In addition, Hodson et al. (1979) report that copper has a deleterious effect on osmoregulation in salmonids. Such an effect in Atlantic salmon could have significant consequences for fish that are routinely moved directly from fresh to salt water and often into cages freshly treated with copper-based paints.

Lewis and Metaxas (1991) measured dissolved copper inside and outside of a freshly treated aquaculture cage in British Columbia, Canada. They report that concentrations of copper inside the cage (0.54 μg/L) were not significantly different from the level outside the cage (0.55 μg/L). The concentrations remained stable for over a month. A sampling station 700 m away had concentrations only slightly lower than those reported at the site (0.38 μg/L). These values are well below the lethal threshold reported here and similar to levels measured in areas where no treated nets were present (Lewis and Metaxas 1991).

In summary, the LC50 of copper to juvenile haddock is 412.5 μg/L over 48 hr. This concentration is much higher than that measured in water in field situations. It seems unlikely that exposure to copper from treated nets will result in acute lethality to young haddock. There remains, however, a considerable amount of uncertainty regarding copper concentrations associated with treatment of nets. The inconsistency of treatment may increase the risk to fish and possibly to other non-target organisms. It is worthy of note that copper is banned as an active ingredient of antifouling paints in Sweden and Holland (Champ 1999).

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