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for Aquarium Fishes and as an Algaecide
in Marine Mammal Water Systems

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Aquarists for years have known that although copper can be lethal, small amounts have a beneficial effect on certain fishes. The use of a penny in a small aquarium tank or a bit of copper screen had long been a practice of some early hobbyists. The utilization of copper in large tanks by professional aquarists did not come about until some 22 years ago when Marineland of Florida became plagued by many fish ectoparasites, as well as considerable amounts of algae. There was a need for some means of combating parasites and algae. As a result of this need, Max Gelfand (1946) started his investigations of the use of copper in water systems. He tested various copper-complexing agents in order to discover a means of preventing copper precipitation in alkaline water. By adding appropriately selected chemical compounds (sequestering agents) to alkaline water, he found that the copper ions were maintained in solution. At pH values as high as 10.5, copper ions remained in solution and proved to be effective in removing algae and certain parasites from the water.¹

Independently, research at the Steinhart Aquarium (Dempster, 1955) led

¹ Gelfand's discovery was patented by the Department of Commerce, U. S. Patent Office (no. 2,400,863, May 21, 1946). On 1 April 1949 he assigned the entire right, title, and interest in his invention and patent to Black Laboratories, Inc., Gainesville, Florida, which on the same date assigned the entire right, title, and interest to Marine Studios, Inc., Marineland, Florida. The method for the use of copper compounds sequestered with aliphatic hydroxy acids in water treatment was kept highly secret for many years; consequently this method of algae and disease control in alkaline water was not available to aquariums other than Marineland.

to the development of a copper-citric acid solution that is stable in sea water. The ratio of 3 parts of copper sulfate pentahydrate to 2 parts of citric acid monohydrate by weight has proved satisfactory. This copper-citric acid solution is used in the treatment of diseased fish.

The success of this therapy suggested the use of sequestered copper as a means of controlling algae in marine mammal tanks, and parasites in fish tanks.

Copper compounds must be utilized with considerable caution since they are toxic not only to animal parasites and algae but also, in varying degrees, to all forms of life. Therefore, when copper is added to water circulating through fish tanks or to water circulating through marine mammal tanks, its toxic effect on the animals must be seriously considered.

There is wide variation among the animals and plants in their tolerance to copper. Most forms of algae and diatoms have a greater degree of tolerance to it than do the vast majority of fishes; consequently, a lethal copper concentration for these phytoplanktonic organisms will most likely be lethal to fish. Thus it is impractical and could be disastrous to use copper as an algacide in fish tanks. Because of the high sensitivity of sea anemones, starfish, nudibranchs, and other invertebrates to copper, its use is precluded in aquarium tanks containing these forms. Marine mammals appear to tolerate copper concentrations up to 1 ppm. Most forms of algae succumb at a concentration of this level; therefore, copper compounds may be used successfully as an algacide in water systems housing marine mammals if the copper is sequestered.

Fishes vary considerably in their ability to tolerate copper. The squirrel-, goat-, turkey-, and some of the butterfly-fishes are the first of the marine forms to show signs of distress when the copper concentration exceeds 0.2 ppm. It has been the experience at the Steinhart Aquarium that most marine fishes will tolerate a concentration of 0.15 ± 0.05 ppm. for a 10-day treatment period. However, 0.2 ppm. should be considered the upper limit, and the concentration should not fall below 0.1 ppm. or the effectiveness of the treatment will be diminished. Even though some fishes may not exhibit signs of distress when the copper concentration exceeds 0.2 ppm. it is neither advisable nor necessary to subject them to higher concentrations, as pathogenic flagellates and ciliates will succumb to the lower concentrations when treatment is extended over the ten-day period.

Although the copper treatment is primarily used as a cure for gill diseases caused by dinoflagellates of the genus *Oodinium*, the Steinhart experience has been that many other fish diseases caused by protozoan organisms respond to this therapy. It may also be used successfully as a prophylactic measure to counteract diseases introduced by newly arrived wild fishes. Treatment with this chemical for one week will free the fish of many of their external protozoan parasites.

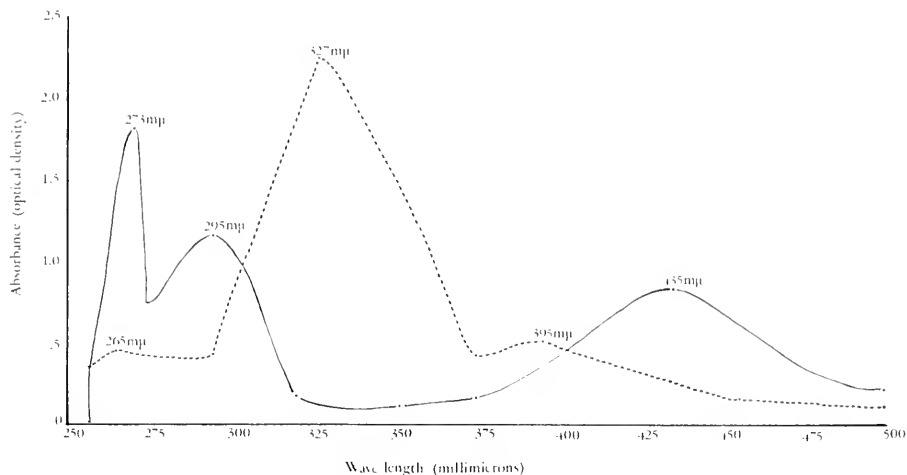


FIGURE 1. Absorption spectra of copper diethyldithiocarbamate (solid line) and nickel diethyldithiocarbamate (broken line) in carbon tetrachloride at concentrations of 1.4 and 1.3 ppm, respectively.

In aquarium water systems where newly collected tropical marine fishes are regularly introduced, it is advisable to treat the water routinely with copper once a month. This will substantially reduce the incidence of disease in aquarium tanks. After each copper treatment, water from the aquarium system should be gradually renewed with fresh sea water and the filters should be thoroughly cleaned.

Care must be taken to reduce normal brine shrimp feeding when the water is being treated with copper. At Steinhart Aquarium heavy mortality has often occurred among small fishes after they have gorged themselves with brine shrimp in copper-treated water (Herald and Dempster, 1965).

When this therapy is to be initiated, copper tests should be made twice daily during the period of copper sulfate addition. Once the concentration of the water system is adjusted, sequestered copper must be slowly added at a rate sufficient to maintain the concentration.²

After the desired therapeutic concentration has been established and the copper concentration is being maintained, daily tests are sufficient. Copper tests may be made by a standard colorimetric method (Snell and Snell, 1954, pp. 107-112). This method utilizes sodium diethyldithiocarbamate as a chromagenic

² Grams of copper sulfate to produce a desired copper concentration may be obtained by the following:

$$\text{CuSO}_4 \cdot 5\text{H}_2\text{O} = \frac{V \times 14.875 \times \text{PPM}}{1000}$$

V = Total water volume in gallons.

PPM = Desired copper concentration.

14.875 = A constant.

agent. As can be seen in figure 1, copper has a broad absorption peak in the visible region, making measurements possible at 437 ± 3 millimicrons. Of the metals that form complexes with the reagent, iron and nickel are the only ones probable in an aquarium system. The iron interference is eliminated by the use of the citrate buffer. Figure 1 clearly shows that very large amounts of nickel must be present to cause an error in the copper determination, since its absorbance in the region of measurement is negligible. Hence sublethal concentrations can be ignored. The details of the procedure used at the Steinhart Aquarium are given below.

NECESSARY REAGENTS:

Sodium diethyldithiocarbamate, reagent grade.

Copper-free water (double distilled from an all-glass still or de-ionized).

Ammonium hydroxide, concentrated reagent grade.

Ammonium hydroxide, 10 percent.

Carbon tetrachloride, reagent grade.

Citric acid, reagent grade.

Copper sulfate pentahydrate, reagent grade.

REAGENT PREPARATION:

Dissolve 1 gm. of $N(C_2H_5)_2 CS_2Na$ in 100 ml. of copper-free water and add 10 percent NH_4OH until the pH reaches 9.6–10 (2 or 3 drops are sufficient).

Store in a bottle of dark glass protected from sunlight.

The indicator treated thus will remain stable for approximately 40 days.

Copper standard solution: Dissolve 0.393 gms. of reagent grade $CuSO_4 \cdot 5H_2O$ in 1 liter of copper-free water. Dilute 10 ml. to 1000 ml. One ml. of this solution contains 0.001 mg. of copper.

Ammonium citrate buffer 20 percent: Dissolve 200 gms. citric acid in approximately 500 ml. of copper-free water.

Add concentrated ammonium hydroxide until the pH reaches 9–9.2 (approx. 240 ml.). Add copper-free water to 1000 ml.

PROCEDURE. Obtain a 100 ml. water sample. Transfer the sample to a separatory funnel, add 5 ml. of ammonium citrate buffer, and mix. Add 1 ml. of sodium diethyldithiocarbamate indicator and mix again. The carbamate, when introduced into the water sample containing copper, produces an amber color. The intensity of this color is proportional to the amount of copper present. After the liquid in the funnel is thoroughly mixed, 10 ml. of carbon tetrachloride is added and the sample is shaken for 2 minutes to extract the copper. In order to achieve complete phase separation the solution must be allowed to stand for 10 minutes. The colored layer is then drained off into a test tube and read in the colorimeter at a wave length of 437 ± 3 millimicrons against an appropriate blank.

A working curve is constructed by analyzing known amounts of copper and

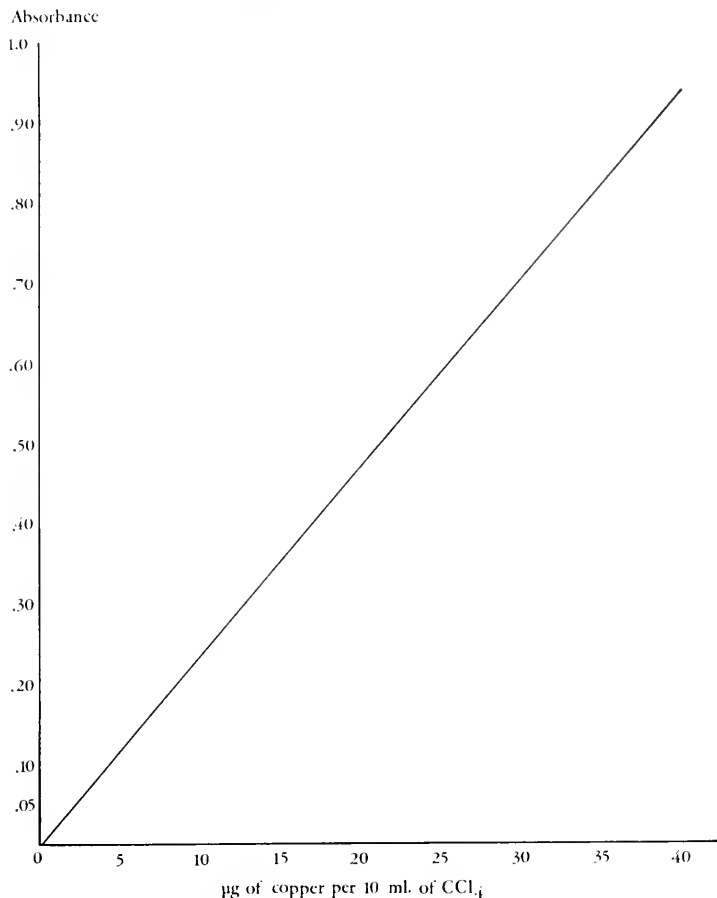


FIGURE 2. Graph for determining copper concentrations in known volumes of water. Absorbance = $0.023 (\mu\text{g Cu}) + 0.005$, $\mu\text{g Cu} = (A - 0.005)/0.023$. The equation of best fit was derived by linear regression.

plotting absorbance (optical density) as the ordinate and micrograms of copper as the abscissa (fig. 2). The line of best fit can be derived by linear regression.

It has been our experience that Beer's law is obeyed over the range of 5 to 40 micrograms copper in 10 ml. of carbon tetrachloride; therefore, the amount of water sampled must be of sufficient volume to contain copper in these amounts.³ In order to maintain a constant extraction volume in all samples, the total volume of each sample should be increased to 100 ml. with copper-free water before it is processed.

³ To convert weights versus volume to ppm. of copper use the following equation:

$$\text{ppm. Cu} = \frac{\text{micrograms Cu}}{\text{ml. water sample}}$$

For the past 15 years copper sulfate has been periodically added to the tropical sea water system at Steinhart Aquarium and as a consequence there has been no epizootic outbreak of disease among the fish in this system since the *Oodinium* attack in 1951. The development of the copper treatment technique has been a boon to the aquarium world. Many of the reef fishes that ordinarily succumb to diseases that attack them when they are kept in captivity, are maintained for long periods in good health as a result of a working knowledge of this technique. Maintaining copper concentrations ranging up to 1 ppm. has been an asset to the marine mammal display at the Aquarium. Concentrations at this level have served as a deterrent to the growth of algae in the water circulating through this water system, and as far as is known copper in this concentration has no ill effect on the animals.

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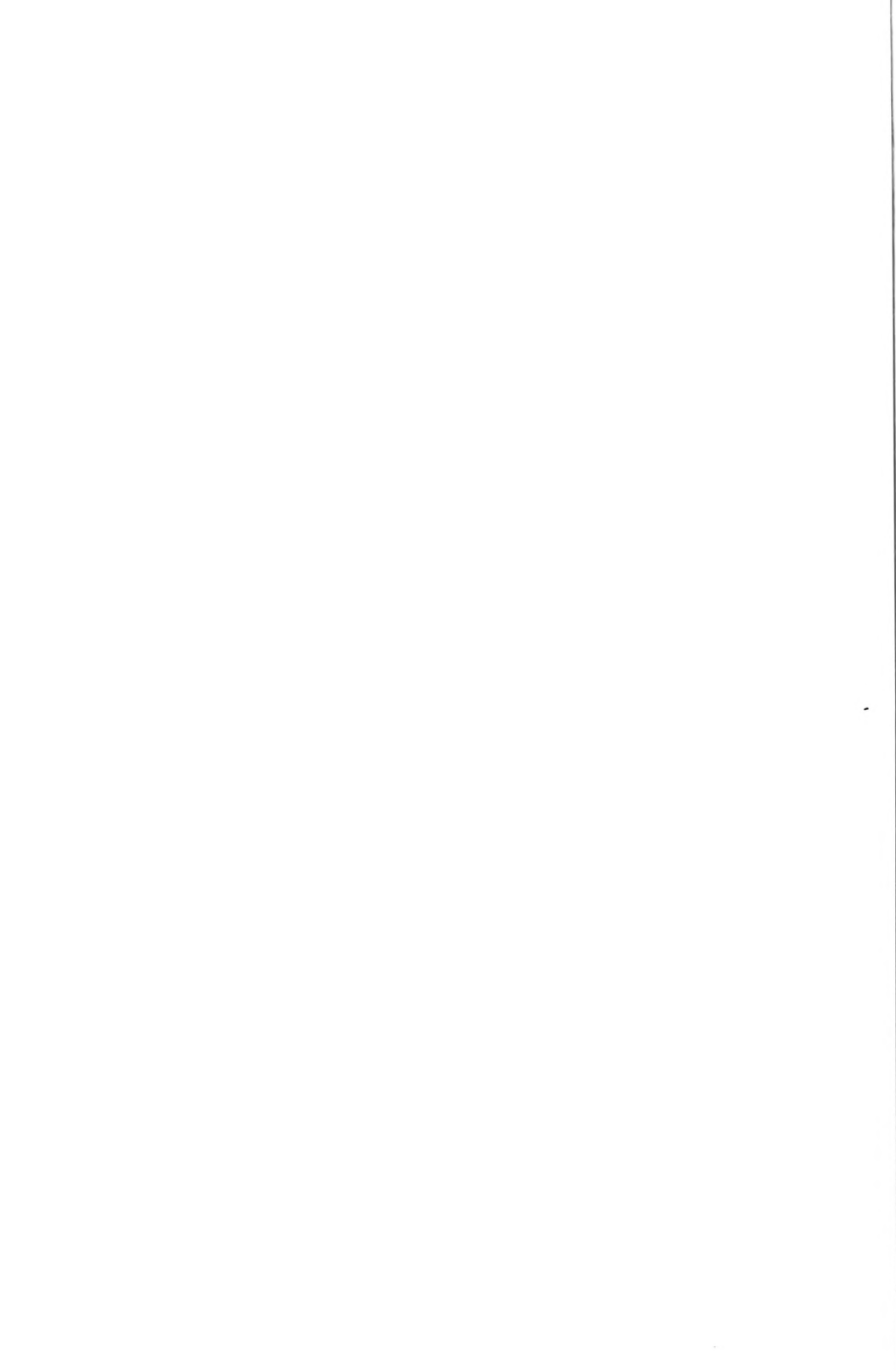
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