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Baseline

Effect of copper on fertilization success in the reef coral *Acropora surculosa*

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Copper, a heavy metal, is an essential element to all living things, but may be toxic to some marine organisms at low concentrations (Heslinga, 1976; Coglianese and Martin, 1981; Mance, 1987; Ringwood, 1992). There is little available information on the toxicity of copper to corals. The effect of copper on corals is of paramount concern because there are numerous sources that expose corals to copper. Copper is a major component of antifouling paints (Claisse and Alzieu, 1993), is found in sewer discharges (Pastorok and Bilyard, 1985), is a component of some fungicides and herbicides that are used on coastal agricultural crops (Cremlyn, 1979), is used to treat wood used as construction materials for coastal waterfront structures (Brown and Eaton, 2001), and is used in heat exchangers in power plants (Stupnisek-Lisak et al., 1998). The concentration of copper in relatively pristine marine environments occurs between 0.01 and 0.03 μg/L (Sadiq, 1992). Given its wide use, copper poses a potential threat to many marine organisms (Fang and Hong, 1999; Brown, 1987; Mance, 1987; Schmidt, 1978). Evaluating its effect on corals is vital in reef management and conservation.

Many researchers have attempted to demonstrate the effects of copper on reproductive success of some species of corals such *Favites chinensis* and *Platygyra ryukunensis* (Heyward, 1988), *Goniastrea aspera* (Reichelt-Brushett and Harrison, 1999), and *Acropora millepora* (Negri and Heyward, 2001). These studies demonstrate that copper has an inhibitory effect on fertilization success in a few species of spawning corals. Further investigation is needed to evaluate the effects of copper on other important reef-building spawning corals over a range of relevant concentrations.

A stock solution of copper (4 mg/L copper = 15 mg $CuSO_4$ in 1 L of deionized H_2O) was prepared and analyzed by atomic absorption spectrometry (AAS) against calibration standards to determine the exact concentration of copper in solution. From this standard solution, the different concentrations of copper tested in this experiment were prepared by diluting with appropriate amount of seawater. Actual concentration was measured at $t = 0$ and at $t = 12$ h during the toxicity test for the 12 h exposure experiment.

Acropora surculosa is a common reef-building coral found on the shallow reef areas around Guam. The reef systems around Guam are mainly fringing reefs close to shore and therefore, this species of corals may have a greater chance of being exposed to pollutants entering from land source. This species of Acropora is fairly easy to identify from other species, spawns very early in the night (19:00 h), and produces lots of gametes that makes it an ideal species to be used for coral toxicity test.

On Guam, coral spawning occurs between the 4th and the 10th nights following each of the full moons of June through August (Richmond and Hunter, 1990). Gravid colonies of *A. surculosa* were collected at least one week prior to the predicted coral spawning periods in June and July. Colonies were maintained in aerated flow-through seawater tanks in the laboratory for the duration of the experiment.

Two gravid colonies were chosen for toxicity test on fertilization, sperm from one colony was used to fertilize eggs from the other (out-crossing). Toxicity tests on fertilization were carried out in 50 mL glass jars with screw-on lids. Each jar contained 30 mL of the desired concentration of copper solution and 16 egg–sperm clusters (8 from each colony), which yield ~80 eggs. Six replicate jars were used for each treatment and for the control. Jars were covered with lids and gently agitated by hand every hour for 3 h

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to help break apart the clusters and allow fertilization to occur.

Toxicity tests were performed at the ambient air temperature of ~28 °C for 5 h to allow for maximum fertilization and early cell cleavage (Heyward, 1988). The development of fertilized eggs was terminated by adding 1 mL of a fixative (10 g/L sodium â-glycerophosphate, 4% formaldehyde buffered at pH 7) which maintained embryo integrity (Negri and Heyward, 2001). Eggs and embryos were assessed for fertilization the following morning under a dissecting microscope. Experiment was assessed based on the method described in Negri and Heyward (2001).

Another toxicity test exposed gametes to the different concentrations of copper for 12 h. This test essentially measure how many gametes survives into the embryo state at the first 12 h of exposure to copper. The 5 h exposure test simply measures the fertilization rate and does not show what happens if the fertilized egg continues to be exposed to potential pollutant. The threshold level may be lower than when it is exposed for a longer period of time.

The numbers for fertilized eggs, unfertilized eggs and mortality were converted to percentages. Arcsine transformed fertilization and mortality data were analyzed using one-way ANOVA followed by Tukey–Kramer post-hoc test with a 95% confidence limits to examine differences between means. Probit analysis was carried out to calculate the 5 h and the 12 h EC_{50} for fertilization (NCSS, 2000).

Fertilization rate after 5 h was 99% in the control (Fig. 1). There was a significant effect of copper on mean fertilization rate for all copper treatments. Copper reduced mean fertilization to 90% at the lowest copper concentration (10 μg/L) tested, a rate that was significantly different from the control. Fertilization rates were reduced to <20% and <10% in 100 and 200 μg/L, respectively. There was no significant difference in mean fertilization rate between the two highest concentrations of copper.

There was a significant effect of copper on gametes that survived the 12 h exposure toxicity test. No gametes survived the 12 h exposure into the embryo stage at concentrations above 58 μg/L (Fig. 2). Less than 50% of gametes survived the 12 h exposure at 12 μg/L. The 12 h EC_{50} is almost four times lower than the 5 h EC_{50} (Table 1).

The initial measured copper concentration in each treatment was between 10% and 15% more than the calculated value based on the copper stock solution (Table 2). After 12 h, there was about a 52–65% recovery of copper in all concentrations, except in the 10 μg/L solution, where 90% of dissolved copper was recovered. This suggest that at concentration above 10 μg/L much of the copper precipitate out of solution.

These are the first quantitative data which show that relatively low concentrations of copper in seawater can disrupt reproductive success in a reef coral from Guam's reefs. These data show similar trend with data from Heyward (1988) in Japan and Reichelt-Brushett and Harrison (1999) and Negri and Heyward (2001) in Australia on other coral species. Heyward (1988) found that concentrations below 100 μg/L did not reduce fertilization but concentration at 100 μg/L reduced fertilization success to less than 50% in gametes from *F. chinensis*, however in *P. ryukyuensis* there was almost no fertilization. Reichelt-Brushett and Harrison (1999) found that fertilization success in *G. aspera*

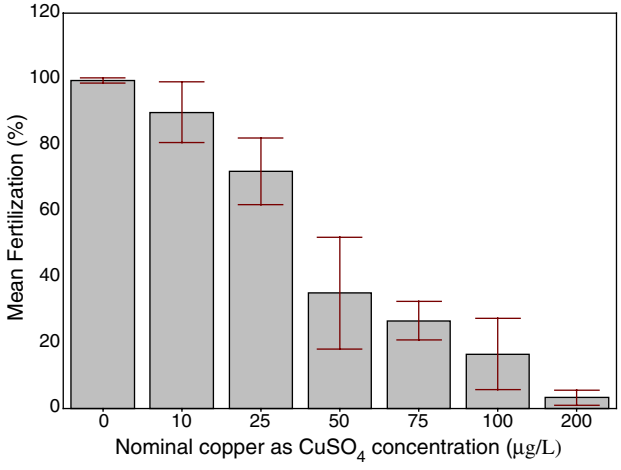

Fig. 1. Effect of copper on fertilization rates (mean ± 0.95 confidence interval) on gametes from *Acropora surculosa* after 5 h exposure.

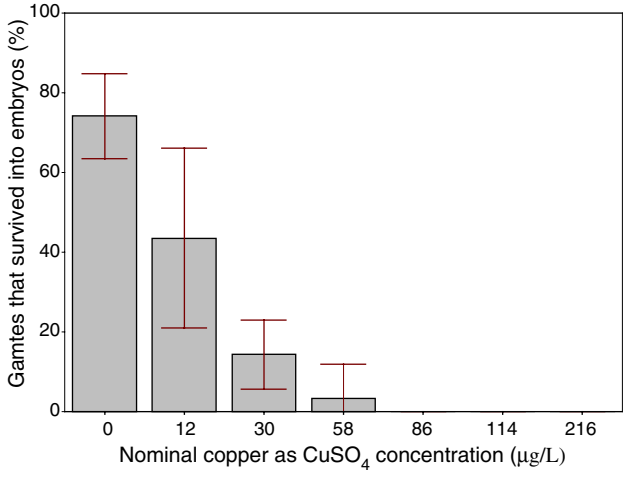

Fig. 2. Effect of copper on number of gametes (mean ± 0.95 confidence interval) that survive into the embryo stage during the first 12 h of exposure.

Table 1
Nominal and measured concentration of copper stock solutions used to make the different copper concentrations for the toxicity tests

Nominal	Measured			
Concentration (μg/L)	0 h	Std. dev.	12 h	Std. dev.
0	0.575	0.500	0.150	0.100
10	12.10	1.192	9.4	0.216
25	30.10	3.623	16.23	4.512
50	58.58	3.028	26.03	8.229
75	86.48	5.963	45.53	4.011
100	114.82	2.696	65.13	8.180
200	216.65	14.266	112.68	39.328

Table 2
Comparison of EC_{50} of copper on variety of marine species

Author	Species	EC_{50} (μg/L)	Exposure time
Reichelt-Brushett and Harrison (1999)	Coral (*Goniastrea aspera*)	14.5	0.5 h exposure of eggs and sperm separately and then combined for fertilization
Negri and Heyward (2001)	Coral (*Acropora millepora*)	17.4	4 h exposure of eggs and sperm after separation
This study	Coral (*Acropora surculosa*)	45.2	5 h exposure of gamete bundles
This study	Coral (*Acropora surculosa*)	11.4	12 h exposure of gamete bundles
Ringwood (1992)	Sea urchin (*Echinometra mathaei*)	14	1 h exposure of sperm
Ringwood (1992)	Bivalve (*Isognomon californicum*)	55	1 h exposure of sperm

was significantly reduced to 41% in 20 μg/L and <1% in 200 μg/L. Negri and Heyward (2001) found that at 17.4 μg/L concentration of copper, fertilization success in *A. millepora* was reduced to 50%.

Furthermore, the 12 h exposure data shows that duration of exposure to copper will have a big negative impact on the number of potential larvae that may be produced from the fertilization process. These data have ecological relevance because in areas of low circulation, gametes may be exposed to potential pollutants for a longer period of time. This would have important bearings on establishing threshold limits for certain pollutants. The previous studies mentioned above all have used short term exposure regimes to calculate threshold limits of copper thus such limits would not accurately predict the potential negative impact on coral population in areas of low circulation.

The differences in the toxicity of copper to coral gametes may result from species differences in sensitivity to copper and/or the variation in experimental methodology. The toxicity response of gametes in this study is three times lower than previous studies that have exposed gametes from other coral species to copper (Table 2).

In this study gametes were exposed to copper and allowed to separate and fertilize as gamete bundles, simultaneously. In previous studies gamete bundles were separated into eggs and sperm. Eggs and sperm were then exposed to copper separately, and combined in the test container for fertilization (Negri and Heyward, 2000; Reichelt-Brushett and Harrison, 1999). This method would have allowed for longer handling of gametes, which may stress them. Stressed gametes may be more susceptible to additional stress, such as addition of pollutant to their environment. In this toxicity test the handling artifact was minimized by combining gametes immediately after they were spawned and placed into the test container with the copper solution.

The type of method used could have had an effect on sensitivity of gametes to copper's toxicity effects. Toxicity of copper may be reduced because when eggs/sperm bundles are exposed to copper, the sperm are inside the egg cluster. The copper may adhere to the eggs, thereby lowering the concentration at which the sperm may be exposed to when the bundles break apart.

Further literature review showed that toxicity of copper to aquatic organisms is related to the copper free ion (Eriksen et al., 2001; Crecilius et al., 1982; Young et al., 1979). However, copper may be complexed to carbonate and hydrogen ions (Pagenkopf et al., 1974) and organic matter (Bately and Gardener, 1978). Its toxicity will therefore be reduced in seawater and in the presence of organic matter, such as eggs. However, in this study where filtered seawater was used, the toxicity of copper to *A. surculosa* gametes was still lower than experiments that have used unfiltered seawater that exposed eggs and sperm to pollutants separately and combined later for fertilization. Table 1 shows that even in the presence of filtered seawater, there is about 35–50% reduction of copper in solution. All data seems to suggest that *A. surculosa* gametes are much less sensitive to copper than *A. millepora* and *G. aspera* (Table 2).

Copper has been shown to damage sperm of other marine species through oxidative stress (Lloyd et al., 1997) but this mechanism has not been demonstrated in corals. This mechanism should be further investigated to determine whether direct exposure of sperm to copper would have had an effect on the toxicity of copper. This may help in separating out the effects due to variation in methodology and the actual species differences in sensitivity to copper.

The mechanism of copper toxicity for coral gametes is not yet clearly understood. It has been suggested that toxicity may predominantly affect the sperm cells. Therefore, future research should expose sperm cells to copper and combine the sperm with clean eggs for fertilization. The opposite should be done to determine whether the exposure of eggs alone will have any effect on the fertilization process. This should also be done with both filtered and unfiltered seawater to determine the adsorption effects of particulates in sweater to the toxicity of copper to gametes.

The results of this study may have broad implications for coral reef conservation. Protecting coral reefs may not be possible without simultaneously controlling the effect of land-based pollution. Copper may enter into the sweater from various sources on land, such as copper mining and sewage discharges. Therefore, it is critical to implement copper monitoring in coral reef areas where there are discharges to evaluate the levels of copper in sweater. Because there is a potential for elevated copper in seawater that would have negative impact on coral population by reducing fertilization success and subsequent larval maturation to limit larval supply that may reduce recruitment of corals juvenile.

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