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Comparison of the Effects of Copper on Respiration and Its Accumulation in Tissue in the Hard Clam *Meretrix lusoria*

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I-Ming Chen (1995) Comparison of the effects on respiration and accumulation in tissue of copper in the hard clam *Meretrix lusoria*. *Zoological Studies* **34**(4): 235-240. A sensitive monitoring system was set up to continuously measure dissolved oxygen concentrations in order to study the effects of copper toxicity on the hard clam, *Meretrix lusoria*. Respiration in *M. lusoria* was repressed within 1 hr after adding the hard clams to sea water containing copper concentrations greater than 14 ppb at both 25°C and 30°C. Liver tissue of *M. lusoria* began to accumulate copper within 24 hr, when the hard clams were submerged in sea water at 30°C with a concentration of 18.6 ppb copper. However, the liver did not accumulate copper within 14 d, when the hard clams were treated with either 15.4 or 17.3 ppb copper. Neither foot nor gill tissue accumulated copper within 14 d for treatments with 15.4, 17.3, and 18.6 ppb, respectively. The results indicate that continuous measurement of respiration was more efficient in revealing the copper toxicity in the hard clam than the tissue accumulation test.

Key words: Copper, Continuous recording of respiration method, Bivalvia.

The chronic effects of copper toxicity on aquatic organisms have been studied in four major ways: (1) effects on growth and reproduction (Mount and Stephan 1969, Arthur and Leonard 1970, McKim and Benoit 1971 1974, Lett et al. 1976, Solbe and Copper 1976); (2) effects on behavior and ecology (Sprague et al. 1965, Saunders and Sprague 1967, Cain and Luoma 1990); (3) effects on histology and biochemistry (Fujiya 1960, Baker 1969, Betzer and Yevich 1975, Vijayamadhavan and Iwai 1975, Brown 1976); and (4) effects on physiology (Calventi and Nigrelli 1961, McKim et al. 1970, MacInnes and Thurgerg 1973, Sellers et al. 1975, Wright and Zamuda 1987, Regoli et al. 1992). The first two methods require a fairly long-term monitoring period and experimental conditions are difficult to control. The third method requires scarifying the animals in order to obtain materials for analysis. This procedure may produce results, with artifacts which are not related to any biological process. Physiological studies also have some of the same drawbacks mentioned above, depending upon the cases studied. For this reason, Chen (1994) developed a continuously recording respiration (CRR) method to overcome some of these shortcoming, such as fluctuation errors caused by discontinuous sampling methods.

Copper pollution is a serious problem in Taiwan (Hung and Han 1991). Oysters respond quickly, in terms of respiration changes, to the increase of copper concentrations in their environment (Chen 1994), e.g., respiration decreases significantly in sea water copper concentrations higher than 25 ppb. However, the liver of oysters accumulates copper only when concentrations exceed 30 ppb in sea water, while no accumulation was observed at copper concentrations lower than 28 ppb. Since, the hard clam *Meretrix lusoria* is the second most economically important bivalve being cultured in Taiwan (Chien 1990), it is necessary to compare respiration measurements and the tissue accumulation method in order to achieve accuracy in monitoring enviromental conditions.

MATERIAL AND METHODS

Hard clams *M. lusoria*, with shell length 3.4-4.2

cm, were obtained from an aquacultural farm. After being individually cleaned, the animal was kept in filtered sea water (32-33 ppt) with about 4.0 ppb copper. Algae, *Skeletonema costatum*, cultured in a copper-free medium, were fed to the hard clams. The hard clams were maintained at a particular temperature for more than one week before examination. The salinities of sea water in the tests and during the acclimatization period were the same.

Respiration was measured by an apparatus described previously (Chen and Fang 1986). In brief, aerated water from an aeration tank was pumped simultaneously into both an experimental chamber and a blank chamber. Copper in the form of $CuSO_4$ was added to the aeration tank at the beginning of the experiment. Dissolved oxygen sensors were set up at the outlet of each chamber. The dissolved oxygen (%) in the outlet water of the blank chamber was monitored to assure the consistency of experimental conditions. The flow rate of water circulation was adjusted to 90 ml/min for each chamber. Three hard clams were used for each measurement. They were starved for one day, and their shells were cleaned with H_2O_2. After residual H_2O_2 was rinsed off, the hard clams were placed in filtered sea water for 3 hr to stabilize the organisms. $CuSO_4$ was used to adjust the concentration of copper in the sea water. The actual copper concentrations in each experiment were measured by use of an atomic absorption spectrophotometer. The initial copper concentration of sea water was 3.4 $\pm$ 0.2 ppb. The temperature of the sea water in one group was maintained at 25 $\pm$ 0.5°C and in the other at 30 $\pm$ 0.5°C during the experiments.

In order to compare the data, a relative change of dissolved oxygen concentrations was defined to show the effects of copper on respiratory rates of *M. lusoria*. This value was calculated by using the maximum change of dissolved oxygen concentration after copper treatment and the mean of dissolved oxygen concentrations of the steady state within the acclimatization period,

$$C_r = (-1)(C_m - C_o)/C_o\ (100\%)$$

where C_m and C_o are the maximum dissolved oxygen concentration after copper treatment and the mean dissolved oxygen concentrations of the steady state within the acclimatization period, respectively. By definition, the relative change of dissolved oxygen concentration is proportional to respiratory rates. Respiration is inhibited when the value of C_r is negative, and enhanced when the value is positive.

To measure the accumulation of copper in tissue, hard clams were kept in a 300-l glass tank. In a preliminary experiment, it was determined that the growth of hard clams was not affected whether algae were supplied either 3 hr/d or 24 hr/d. The algae did not accumulate copper even if they were incubated in 100 ppb copper for 3 hr. Therefore in subsequent experiments, cultured algae were fed to the hard clams for 3 hr/d. Unconsumed algae were filtered out. To reduce the accumulation of hard clam excrement in the water, 1/3 of the water volume was changed everyday. The salinity of sea water was kept between 32-34 ppt at a temperature in the range of 30 $\pm$ 0.5°C. The copper concentration in sea water was adjusted with $CuSO_4$ and monitored after each water change. Accumulation of copper in various tissues was examined in hard clams cultivated under three different concentrations of copper for two weeks. Hard clam samples were frozen in liquid nitrogen for 20 s and then separated into liver, gill and foot. The tissues became too brittle for dissecting if the samples were kept in liquid nitrogen more than 30 s. Thereafter, tissues pooled from ten hard clams were dried at 105°C to constant weight. Dried tissues were digested in a mixed solution of HNO_3 and H_2SO_4 under heat. The supernatant was collected for further measurements. Copper in the water samples was chelated with ammonium pyrrolidine dithio-carbomate (APDC), then extracted into methyl isobutyl ketone (MIBK) (APHA et al. 1985), and measured in a flame atomic absorption spectrophotometer (Perkin-Elmer 2380).

RESULTS

The concentration of dissolved oxygen remained at 95% saturated values in the blank chamber for all concentrations of copper up to 1,000 ppb, whereas in the experimental chambers, with three hard clams per set, it remained steady (with small variations) but was lower than in the blank chamber. All experiments were carried out for 3 hr after the copper addition. In almost all cases, the respiration rate reached a steady state within 1 hr. Typically, there were three patterns of hard clam respiration rate response to the effects of copper. The first response pattern (decreased respiration pattern) occurred at high copper concentrations, during which the dissolved oxygen concentration in the outlet of the experimental

chamber increased to a steady value. The response time between the copper addition and the steady state, as shown in Figs. 1a-1c, became shorter as copper concentrations increased. The response times were 20, 27, and 35 min for copper concentrations of 981, 80, and 24 ppb, respectively. The second response pattern (increase respiration pattern) occurred at a copper concentration of 9.4 ppb, during which the dissolved oxygen concentration in the outlet of the experimental chamber decreased after a copper addition (Fig. 1d). The third response pattern (constant respiration) occurred at a copper concentration of 8.0 ppb in which the dissolved oxygen concentration in the outlet of the experimental chamber remained constant (Fig. 1e).

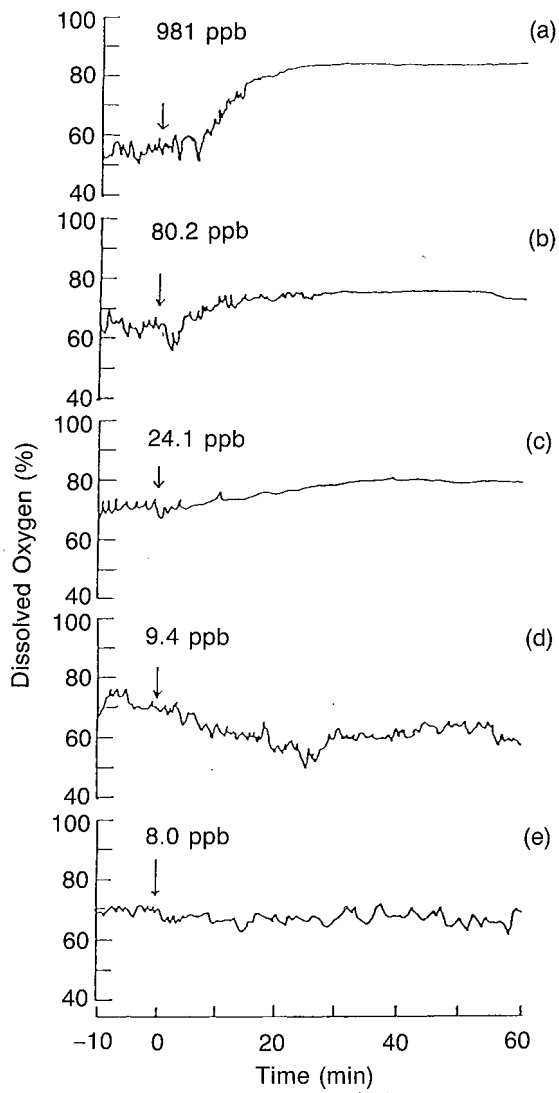

Fig. 1. The influence of copper on the respiration of hard clam, water temperature in 25°C.
Three hard clams were used for each experiment. $CuSO_4$ was added at the time indicated by the arrow. The concentrations of copper were 981 ppb (a), 80.2 ppb (b), 24.1 ppb (c), 9.4 ppb (d), and 8.0 ppb (e), respectively.

As shown in Fig. 2, the C_r values did not change uniformly with respect to copper concentrations at either 25°C or 30°C. The C_r value remained zero until the threshold concentration was reached, then increased to a peak (a positive value), and decreased dramatically to negative values for copper concentrations higher than the critical concentration. The threshold concentration was 8 ppb and the critical concentration was 14 ppb at both 25°C and 30°C.

The concentrations of copper in the aqueous phase for each tissue accumulation test were maintained at 15.4 ± 0.6 (15 ppb set), 17.3 ± 0.5 (17 ppb set), and 18.6 ± 0.6 (19 ppb set) ppb, respectively. The copper concentration of gill tissue in the 15, 17, and 19 ppb sets changed slightly during the first 7 d but remained constant to 14 d (Fig. 3). There was no copper accumulations observed in foot tissue for the 15, 17, and 19 ppb sets. There were some slight up-and-down variations but no accumulation in liver tissue for 14 d in either the 15 or 17 ppb sets, while copper was accumulated rapidly and remained at high levels for 14 d in the 19 ppb set. The 24-hr profiles of copper concentrations in liver are shown in Fig. 4. The concentrations of copper in liver increased gradually in the 19 ppb set, within 24 hr, while there was no accumulation observed for other treatments.

DISCUSSION

It has been proposed that copper enters the body of aquatic animals via two major paths: (1)

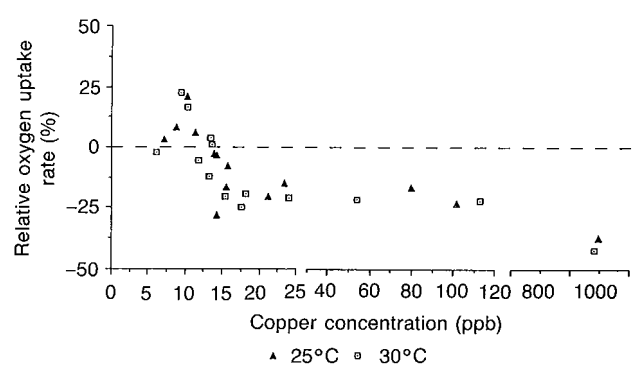

Fig. 2. The profiles of relative oxygen consumption rates in different copper concentrations.

gill or skin and (2) the mouth. Ikuta (1972) suggested that only 1/4 – 1/5 of copper in oysters came from feeding while it primarily came directly from sea water. Hard clams and oysters belong to the same class of Bivalvia; therefore, this study focused on the effects of copper, entering through the gills or skin, on the respiration of the hard clam using the CRR method.

The present results demonstrate that the respiratory rates of hard clams decreased in response to copper environmental concentrations higher than a critical value. This phenomenon has also been observed in mussels (Brown and Newell 1972), mud snails (MacInnes and Thruberg 1973) and oysters (Chen 1994).

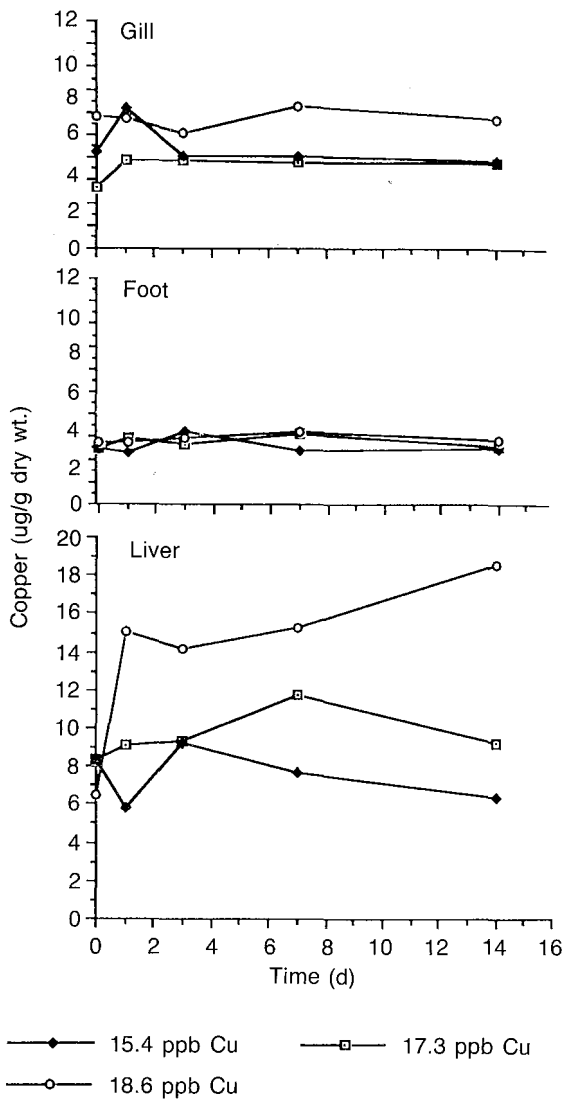

Fig. 3. The concentrations of copper accumulated in different organs of hard clams kept in copper concentrations of 15.4, 17.3, and 18.6 ppb.

The respiratory rates of the hard clam increased when copper concentrations were between 8 and 13 ppb. A similar phenomenon was observed in mud snails and oysters. The respiratory rates of mud snails treated with 0.5 ppm copper were higher than those treated with either 1 ppm or 0.25 ppm (MacInnes and Thurberg 1973). The phenomenon also occurred with oysters at 20-23 ppb copper (Chen 1994). Sellers et al. (1975) observed that rainbow trout increased its behavioral cleaning motions and coughing frequency to get rid of mucus stimulated by a copper load. Therefore, it is suggested that hard clams and oysters might also increase their metabolic rates to supply energy for cleaning motions to remove mucus.

The effects of temperature variations between 25 and 30°C on the sensitivity of hard clams (present results) and oysters (Chen 1994) to copper were negligible. However, the respiratory response times of hard clams to copper toxicity at the critical value were shorter in high concentrations than in low concentrations (Figs. la-1c).

The copper accumulation experiments were carried out at 30°C because sea water temperatures in southern Taiwan can reach 33°C (Hsieh et al. 1991). Among the experimental sets with copper concentrations of 15, 17, and 19 ppb, the liver of hard clams accumulated copper only in the 19 ppb set, and the amount of copper accumulated by

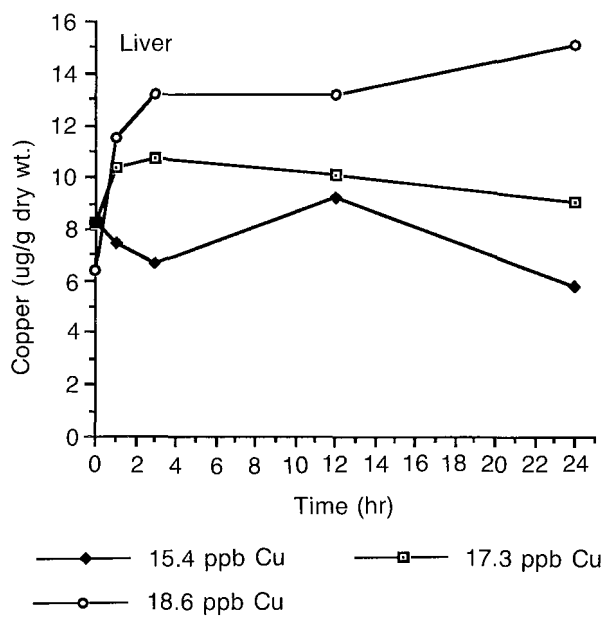

Fig. 4. The concentrations of copper accumulated in livers of hard clams kept in copper concentrations of 15.4, 17.3 and 18.6 ppb for 24 hr.

liver after 14 d was three times higher than the initial amount. The livers of oysters accumulate copper during treatment with 30 ppb, but do not accumulate copper for concentrations lower than 28 ppb (Chen 1994). Therefore, it is concluded that hard clams initiate copper accumulation at a lower environmental copper concentration than oysters.

In this study copper concentration in gills varied within the first day but no accumulation was observed after 14 d. Using ^{64}Cu to determine the copper absorption rate in *Busycon cancaliculatum*, it was found that copper accumulation in the gill reached equilibrium in 1 hr with treatment at 6-9 ppb copper. This means that the transfer rate of copper from water into gill tissue is equal to the rate transferred from gills to other parts of the body in 1 hr. However, when copper concentration was 109 ppb, the accumulation of copper was still increasing after 24 hr (Betzer and Pilson 1975). In this study the accumulation of copper in gills was not significant because the copper was rapidly being transferred to other parts of the body such as the liver. The present results also show that the concentration of accumulated copper in liver increased rapidly within 24 hr.

In addition to tissue accumulation, the tissue osmotic value of hard clams was also affected by copper at a concentration higher than 100 ppb (Chen and Chiu 1991). The continuous recording respiration method had been conclusively shown to be more sensitive than other methods for measuring the effects of copper on hard clam metabolism. Three clams should be used for each experimental set because of instrument limitations with dissolved oxygen measurements. At the critical concentration, the experimental data fluctuated from positive to negative values of C_r due to the variations of individual responses to the critical concentration. This fluctuation was also observed in oysters (Chen 1994).

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銅對文蛤呼吸及組織累積測試之比較

陳 一 鳴[1]

依測試生物呼吸量之減少而使水中溶氧量升高的方式，自設一組連續監測溶氧量變化之系統，測試銅對生物之影響。由文蛤呼吸率測試得知不論水溫在25或30°C銅只要超過14 ppb即會抑制其呼吸。此方法僅需要一個小時即可完成，較組織蓄積量測試方式須要一天以上的時間來得短。蓄積測試得知文蛤在水溫30°C下，海水中銅含量在18.6 ppb，二十四小時內肝臟即有銅的累積。銅在15.4及17.3 ppb海水中經兩週的飼育，肝臟並無累積銅的現象。而在此三組銅濃度下經兩週的飼育，鰓及斧足亦無銅累積之現象。故由連續監測呼吸率方式測試銅對文蛤之影響其敏感度也較高。

關鍵詞：銅，連續監測呼吸方法，二枚貝。

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