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Research Article

Impact of Two Sublethal Concentrations of Copper Chloride and Chlorine on the Excretory Products of Crab *Sesarma quadratum* (*Fabricius*)

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Abstract: Excretion in estuarine animals is considered to be an important factor in assessing their physiological status. The pattern of oxygen consumption and excretion patterns (ammonia and nitrite) in medium size mud crabs of *S. quadratum* (3 to 4 g weight) were studied under laboratory conditions at two sublethal concentrations of 1/10 and 1/3 of the LC_{50} value of copper chloride and chlorine. The rates of oxygen consumption and excreted products were negatively correlated to the sublethal concentrations of the toxicant. Reduced rates of oxygen consumption and excretion of ammonia and nitrite were observed when exposed to these sublethal concentrations.

Key Words: *S. quadratum*, oxygen consumption, excretion, ammonia and nitrite.

Bakır Korid ve Klorin'in İki Subletal Konsantrasyonunun Çamur Yengeci, *Sesarma quadratum (Fabricius)*'un Boşaltım Ürünleri Üzerine Etkisi

Özet: Haliç hayvanlarında boşaltım hayvanın fizyolojik durumunun değerlendirilmesinde önemli bir faktördür. Orta boy (3-4 g ağırlığında) çamur yengeci, *S. quadratum*'da oksijen tüketimi ve boşaltım desenleri laboratuvar koşullarında bakır klorid ve klorinin LC_{50}'larının 1/10 ve 1/3'ü olan iki subletal konsantrasyonunda çalışıldı. Oksijen tüketimi ve boşaltım üretim hızı toksikantların subletal konsantrasyonları ile negatif korelasyon gösterdi. Oksijen tüketimi ve amonyak ve nitrit boşaltımı hızlarının hayvanlar bu subletal konsantrasyonlara maruz bırakılınca düştükleri gözlendi.

Anahtar Sözcükler: *S. quadratum*, oksijen tüketimi, boşaltım, amonyak ve nitrit

Introduction

Since copper is a widely distributed heavy metal, it has been the subject of increasing research activities to determine its incidence and to control its concentration in estuarine and coastal marine habitats. Due to its persistence in the environment, its toxicity at high concentrations and a tendency to accumulate in the biota there is a potential hazard to human health (1). Copper is also an essential trace element required by all marine organisms and yet is one of the most poisonous of heavy metals when present in excess (2). Katticaran and Salih (3) studied the effects of copper on the clam, *Sunetta scripta*. However, studies on the effects of copper on the physiological performance of bivalves other than mussels are scarce. The objective of their experimental study was to investigate the effect of copper contamination on the physiological responses of the clam when chronically exposed to an environmental level of copper and to assess the sensitivity of the physiological approach of detecting deviations from normal performance.

Chlorine is a toxicant which is used in the paper and textile industries and in the production of bleaching power. Such industrial discharges reach the oceans resulting in hazardous consequences. Chlorine is also used as a biocide to control fouling organisms that settle in the water cooling systems of electric power plants. Chlorine bioassays were mostly made with fresh water and estuarine fauna by exposing them to chlorinated discharges from power plants and waste water treatment plants (4). Madras Atomic Power Station (MAPS) uses about 300 kg of chlorine per day to control biofouling and the residual chlorine reaches the coastal water (5). Exposure to chlorinated cooling waters results in reduced oxygen consumption and growth of larval lobsters (6),

and reduced feeding and egg production in rotifers and copepods. The occurrence of reduced levels of oxygen resulted in gill damage in marine fish (7,8).

In this study, experiments were carried out to estimate oxygen consumption and excretion of ammonia and nitrite in the estuarine mud crab *Sesarma quadratum* at two sublethal concentrations of copper chloride and chlorine. All experiments were carried out for durations of 1, 7, 14, 21 days.

Materials and Methods

For physiological studies, healthy male crabs of *Sesarma quadratum* in the intermoult stage were used. Specimens weighing approximately 3 to 4 g were collected from Adyar estuarine region. In the laboratory, about 20 crabs were kept in 20 l of 50% seawater with a salinity of 17 ppt, temperature 27 ± 2.0 °C, pH 8.0 ± 0.1, dissolved oxygen 6.0 ± 0.5 mg/l and in 12 h L/D cycle. The control and experimental crabs, following conventional procedure, were not fed during the experiment period. The LC_{50} was calculated through a probit analysis of log transformed data. The probit line was fitted with log toxicants against mortality and the LC_{50} was calculated where the probit line crossed 50% mortality (9). LC_{50} values for copper chloride and chlorine were estimated to be 28 ppm and 2.95 ppm respectively.

Experiment of sublethal concentrations on physiological responses of oxygen consumption

Oxygen consumption by the organism was determined by the method outlined by Balaji (10) and Sasikumar (11). A closed respiratory chamber of 1 L capacity was used to determine the oxygen consumption by the crab. Initial oxygen concentration of the water samples was determined. Then a medium sized male crab was introduced into the chamber individually and allowed to respire for one hour. The difference between the initial and final readings was taken as the oxygen consumption of the crab. All the experiments were carried out in triplicate with 50% seawater of two different sublethal concentrations of copper chloride 1/10 (2.8 ppm) and 1/3 (9.3 ppm) and chlorine 1/10 (0.29 ppm) and 1/3 (0.98 ppm) for 1, 7, 14 and 21 days.

Experiments on ammonia and nitrite excretion

One litre of seawater was filtered through a glass filter paper (0.42 µ) to remove suspended particles. The crab, *Sesarma quadratum*, of a medium size group was introduced. After 1 h, aliquots of water samples were drawn from the animal chamber and contents of nitrite and ammonia were estimated by following standard analytical methods (12). All experiments were carried out in triplicate with 50% seawater of two different sublethal concentrations of copper and chlorine for 1, 7, 14, and 21 days.

All the values of physiological experiments are given as mean ± standard error. The significance of the difference between the mean values of the control and experimental crabs were analysed using the Two-Way-ANOVA.

Results

Effect of copper chloride on crab

Oxygen consumption

Data on the amount of oxygen consumption in medium-size *Sesarma quadratum* at two different sublethal concentration of copper chloride [1/10 (EX1) and 1/3 EX2] are given in Figure 1. An inhibition in the amounts of oxygen consumption was observed in the crabs after exposure to copper chloride for 1, 7, 14 and 21 days. The results indicated that the crabs consumed less oxygen when exposed to high sublethal concentrations. Oxygen consumption of crabs at two different sublethal (copper chloride) concentrations showed a progressive decrease as the sublethal levels increased. A statistically significant decrease in consumption was observed ($P < 0.01$) for high sublethal concentrations for 21 days.

Excretion of ammonia and nitrite

Data on the excretion level of medium-size *Sesarma quadratum* at two sublethal concentration of copper chloride levels are given in Figure 1. Low levels of ammonia and nitrite excretion were observed at sublethal concentrations of copper chloride. Ammonia and nitrite contents of the crab excreta were estimated after an exposure to copper chloride solution for 1, 7, 14 and 21 days. A significant inhibition in the excretion of ammonia and nitrite was recorded after 1, 7, 14 and 21 days of exposure to the sublethal concentration of copper chloride. The level of excretion of ammonia ($P < 0.01$) and nitrite ($P < 0.01$) was statistically significant for 21 days.

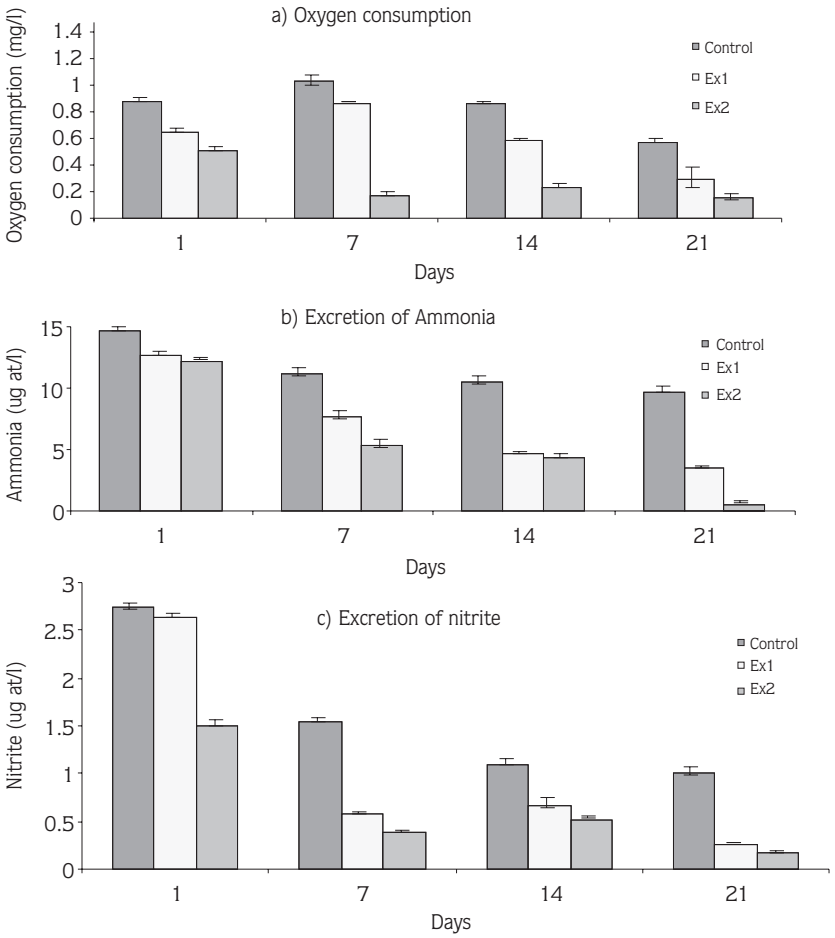

Figure 1. Physiological response of *S. quadratum* when exposed to two sublethal concentrations of copper chloride.

Effect of chlorine on crab

Oxygen consumption

The oxygen consumption of crabs at two different sublethal concentrations of chlorine showed a progressive decrease as the residual chlorine levels increased. Two sublethal concentrations inhibited the amount of oxygen of the crabs during the exposed period of 1, 7, 14 and 21 days. Significant inhibition in the amount of oxygen consumption was recorded after 1, 7, 14 and 21 days exposure to chlorine (Figure 2). Oxygen consumption was statistically significant ($P < 0.01$) for high sublethal concentrations.

Excretion of ammonia and nitrite

When exposed to sublethal concentrations, there was a change in the pattern of ammonia excretion (Figure 2). A minimum amount of ammonia excretion was observed at the sublethal concentration of 0.984 ppm after an exposure of 1, 7, 14 and 21 days. There was a decrease in the excretion of ammonia which was statistically significant $P < 0.01$ for 21 days. The nitrite content was analysed in the crab excreta against the sublethal concentrations. Low levels of nitrite excretion was observed with an exposure of 1, 7, 14 and 21 days at sublethal concentrations. Decreased nitrite excretion was statistically significant ($P < 0.001$) for 21 days.

The effect of copper chloride and chlorine reduced the amount of oxygen consumption in *Sesarma quadratum* as well as ammonia and nitrite excretion at two sublethal concentrations.

Discussion

In the present study, the oxygen consumption of *Sesarma quadratum* was inhibited on exposure to a period of 1, 7, 14 and 21 days, when they were exposed to sublethal concentration of copper and chlorine. Oxygen consumption is a valuable indication of sublethal stress.

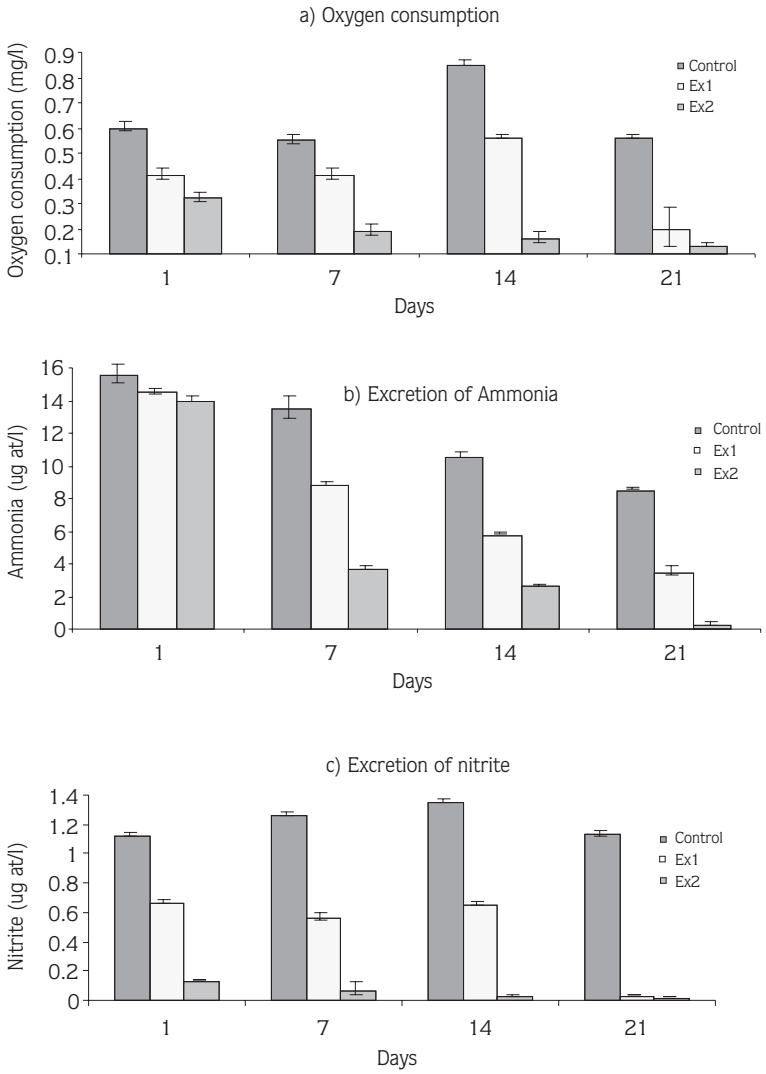

Figure 2. Physiological response of *S. quadratum* when exposed to two sublethal concentrations of chlorine.

Decrease in oxygen consumption appears to be a protective measure to ensure that there is a lower intake of toxic substances (13). Such a decrease in the amount of oxygen consumption may be due to the damage of the respiratory epithelium, enlargement and inhibition of gills, or ATPase activity (14,15).

Many metals have been shown to affect the respiration of various aquatic animals (16-18). Depletion of oxygen consumption and filtration rates in the clams after one-month exposure to xylene, benzene and WSF of gear oil, have been considered to be due to the histopathological damage in gills, which may have resulted in the malfunctioning of gills. Such damage to gills of *Gafrarium divaricatum* was observed when exposed to petroleum hydrocarbons and copper (19). The oxygen consumption of *Perna viridis* (L.) at different concentrations of chlorine showed a progressive decrease while the residual chlorine level was increased (20).

The role of ammonia and nitrite excretion by the marine organisms on the environment and animal physiology have been studied (21). These studies indicated that the excretion of nitrite and nitrate by animals may be an intrinsic mechanism for the detoxification of ammonia in the blood, as well as to maintain electro neutrality inside the animal. Decreased salinity resulted in decreased excretion rates of nitrate and nitrite in *P. viridis* (20).

The excretion of ammonia and nitrite may be regarded in accordance with the rate of protein and amino acid catabolism (22). Faecal matter production of

mussels was inhibited at different residual chlorine levels (20). Similarly, in the present study, the inhibition of ammonia and nitrite levels was observed in *S. quadratum* when they were exposed to two sublethal concentrations of copper chloride and chlorine.

In the light of the ecotoxicological information reported in the present study, the observations made by an earlier author (5) carry much ecological significance. A survey conducted revealed that the intertidal mole crab *Emerita asiatica* has been eliminated in the neighbourhood of the Madras Atomic Power Station (MAPS). This might be due to the input of chlorine into the coastal environment, hence more research should conducted.

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References

1. Paez-Osuna, F., Guerrero-Galvan, S.R., Ruis-Fernandez, A.C.: The environmental impact of shrimp aquaculture and the coastal pollution in Mexico, Marine Pollution Bulletin, 1998; 36 (1): 65-75.
2. Davenport, J., Redpath, K.J.: Copper in the mussel *Mytilus edulis* L. In Toxins, Drugs and Pollutants in Marine Animals (eds.) Bohs *et al*., Springer-Verlag, Berlin, 1984; 176-189.
3. Katticaram, C.M., Salih, K.Y.: Copper induced metabolic changes in *Sunetta scripta* (Bivalvia): oxygen uptake and lactic acid production. Bulletin of Environmental Contamination and Toxicology, 1992; 48: 592-598.
4. Turner, A., Chu, A.: Chlorine toxicity as a function of environmental variables and species tolerance. R.L. Jolley, W.A. Brungs, J.A. Cotruvo, R.B. Cumming, J.S. Mattice and V.A. Jacobs, eds. 4(2). Water chlorination: Environmental Impact and Health Effects. Ann Arbor Science Publishers. Inc. U.S.A. 1983; 927-946.
5. Azariah, J.: Environmental ethics of chlorine in marine biome. Eubios J. of Asian and International Bioethics, 2000; 10: 81-82.
6. Capuzzo, J.M.: The effects of free chlorine and chloramine on growth and respiration rates of larval lobsters (*Homarus americans*). Water Res., 1977; 11: 1021-1024.
7. Davis, W.P., Middaugh, D.P.: A review of the impact of chlorination processes upon marine ecosystems. In the Environmental Impact of Water Chlorination, proceedings, R.L., Jolle (ed.) Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1976; 299-325.
8. Capuzzo, J.M., Davidson, J.A., Lawrence, S.A., Libni, M.: The differential effects of free and combined chlorine on juvenile marine fish. Estuar. Coast. Mar. Sci., 1977; 5: 733-741.
9. Finney, D.J.: Probit analysis. A statistical treatment of the sigmoid response curve. 2nd ed., Cambridge University Press, London, 1962; 318.
10. Balaji, M.: Investigations on bio fouling at two parts in Andhra Pradesh, India and some aspects of toxicity of copper to the fouling bivalve, *Mytilopsis sallei*, Ph.D. Thesis, Andhra University, India, 1988.
11. Sasikumar, N., Azariah, J., Nair, K.V.K.: Sublethal responses of barnacles to chlorine: An experimental study for power plant biofouling control. Mar. Behav. Physiol., 1993; 24: 55-56.
12. Parsons, T.R., Maita, R., Lalli, C.M.: A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York, 1984; 1-173.
13. Sultana, R., Uma Devi, V.: Oxygen consumption in cat fish, *Mystus gulio* (Ham 1, exposed to heavy metals. J. Environ. Biol., 1995; 16 (3): 207-210.
14. Walshe, T.M.: Wilsons disease, a review. In: The Biochemistry of Copper eds. by J.P. Pesiach, P. Aisen and B.E. Blumberg), Academic Press, New York, 1996; 588.
15. Shivaraj, K.M., Patil, H.S.: Oxygen consumption in *Lepidocephalicthys guntea* following exposure to sublethal levels of cadmium and cobalt. Proc. Nat. Symp. Anim. Met. Poll., 1988; 109-191.
16. Skidmore, J.F.: Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulfate. Exp. Biol., 1970; 52: 481-494.
17. Brown, H.E., Newell, R.C.: The effect of copper and zinc on the metabolism of the mussel *Mytilus edulis*. Mar. Biol., 1972; 16 (2): 108-118.
18. Vernberg, W., Vernberg, J.: The synergistic effects of temperature, salinity and mercury on survival and metabolism of the adult fiddler crab, *Uca pugilator*. Fish. Bull., 1972; 70 (2): 415-420.
19. Tendulkar, S.P.: The effects of oil and some petroleum hydrocarbons on the inter-tidal clam *Gafrarium divaricatum* (G melin), Ph.D. Thesis, University of Bombay, India, 1996.
20. Masilamani, J.G.: Studies on biofouling and its control in nuclear power plant with special reference to lethal and sublethal effects of different antifouling techniques on green mussel *Perna viridis* (L), Ph.D. Thesis, University of Madras, Tamilnadu, India, 1998.
21. Chen, C.J., Chen, C.T., Eheng, S.Y.: Nitrogen excretion and changes of haemocyanin, protein and free amino acid levels in the haemolymph of *Penaeus monodon* exposed to different concentration of ambient ammonia-N- at different salinity levels. Mar. Ecol. Prog. Ser., 1994; 110: 85-94.
22. Widdows, J.: Physiological indices of stress in *Mytilus edulis* (L). J. Mar. Biol. Assoc., U.K. 1978; 58: 125-142.