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# Changes in oxygen consumption and heart rate of the blue swimming crab, *Portunus pelagicus* (Linnaeus, 1766) following exposure to sublethal concentrations of copper

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***Abstract:*** *Weight specific oxygen consumption and heart rates were investigated in blue swimming crab, Portunus pelagicus to indicate the stress in response to increasing salinities (50, 75, 100 and 125%SW) and increasing copper concentration (0, 0.75, 1.5, 3 and 6 ppm copper) at temperature 25 ºC. Oxygen consumption ($\mu mol.g^{-1}.h^{-1}$) was measured using closed system respirometer (Strathkelvin Instruments oxygen meter model 781). Heart rates ($beat.min^{-1}$) were recorded using impedance techniques. It has been found that oxygen consumption and heart rate increased with decreasing salinities and increasing copper concentration. The statistic showed weight specific oxygen consumption of P. pelagicus after 96 hr exposure to 6 ppm copper in all salinities were significantly different (P<0.05) from 0 ppm copper. Mean heart rate of P. pelagicus in 50%SW after 96 hr exposure to 6 ppm copper were significantly different (P<0.05) from 0 ppm copper.*

***Key words:*** *Portunus pelagicus, Oxygen consumption, Heart rate, Copper.*

## Introduction

Pollution of the marine environment with copper from anthropogenic sources is most likely to occur in the coastal zone and estuaries (McLusky *et al*., 1986). It is estimated that between 35 and 90 kt of copper have been annually discharged into aquatic ecosystems (Nriagu and Pacyna, 1988). US EPA (Keith and Telliard, 1979) found that copper being a prime constituent (55%) of heavy metal in analyzed water samples. Copper is used as molluscicide in many countries to control the snail intermediate host of the trematode parasite, *Schistosoma* (Wolmarans and Yssel, 1988).

Copper is both a toxic compound and an essential metal in animals. This is particularly true in crustaceans, where huge amounts of copper are components of the active site of a very abundant protein, the respiratory pigment hemocyanin. In the shore crab, *Carcinus maenas* (L.), the acute toxic action of water-borne copper appears directed mainly toward the gill epithelium, leading to important cytological damage to gill tissue (Nonnotte *et al*., 1993). Several other studies have arrived at similar conclusions that essential functions of the gill, gas exchange, is severely affected, as indicated by a marked decrease of partial pressure of oxygen in hemolymph (Spicer and Weber, 1992). It has been noted that the toxicity of copper for estuarine animals increases with decresing salinity (Bjerregaard and Vislie, 1986).

It is generally accepted that oxygen consumption ($Mo_2$) gives a good indication of the overall metabolic state of an animal and also is a useful indicator of sublethal physiological effects of heavy metal poisoning. Oxygen consumption and heart rate of crabs were reported to increase in response to a reduction in salinity (Dimock and Groves, 1975; Gilles, 1973; Taylor, 1977; Taylor *et al*., 1977; Chen and Chia, 1996; Davenport *et al*., 1980). Spicer and Weber (1992) reported that heavy metal such as copper generally reduce oxygen consumption and disrupt cardiac and respiratory activity (Depledge, 1984). However, Vosloo *et al*. (2002) found that copper-exposed crabs showed a significant increase in oxygen consumption after 7 and 14 days, but decreased significantly by 40% after 21 days. The decrease in oxygen consumption is probably caused by ultrastructural damage to gill epithelium (Lappivaara *et al*., 1995).

The blue swimming crab, *Portunus pelagicus* supports substantial commercial fisheries in Thailand. They are found in nearshore marine and estuarine waters throughout the Indo-West Pacific (Stephenson, 1962; Kailola *et al*., 1993). Compared with a vast literature on the respiratory physiology of the Brachyura, the information available for the Portunid crab is restricted to only a few species such as *Carcinus maenas* (Depledge, 1984; Bamber and Depledge, 1997; Hebel *et al*., 1999), *Cancer irroratus* (Thurberg *et al*., 1973) and *C. pagurus* (Spicer and Weber, 1992). Most studies have been conducted on the shore crabs. The present study was therefore undertaken to measure oxygen consumption and heart rate of *P. pelagicus* as an indicator of stress in response to salinities and copper concentrations. This information was possible to determine to what extent acute exposure to sublethal concentration of copper would be detrimental to physiology of this crab species.

## Materials and Methods

**Maintenance of crabs:** Adult males of the blue swimming crab, *Portunus pelagicus* weighing between 10-30 g with an average of 17±0.98 g were obtained from fishermen at Bangsaen, Chonburi province, Thailand. Crabs were transported to laboratory in the Department of Aquatic Science, Faculty of

science, Burapha University. They were held in fiber glass tanks with a recirculating seawater system at salinity of 35 ppt (=100%SW) under natural dark-light cycle. Crabs were acclimated for at least one week prior using in the experiments and fed every 2 days with pieces of mussel. Crabs were not fed 48 hrs before and during the experimental periods.

**Exposure of crabs to copper:** Experimental glass chambers (12 x 14 x 14 cm) were necessary to be absorbed in copper before experimental tests. They were acid-washed and then rinsed twice with distilled water, before being filled with the appropriate copper ($CuCl_2$) contaminated seawater. Water was then aerated and left for 7 day to allow the copper to be absorbed onto the sides of the chamber. After that, water was replaced and crabs were introduced into each chamber. Each chamber allowed the exposure of one crab at a time, and ensured that animal was submerged at all times and that exposure conditions were the same for all animals. Crabs were exposed to 0, 0.75, 1.5, 3 and 6 ppm copper in 50, 75, 100 and 125%SW for 96 hr.

**Measurement of oxygen consumption:** After 96 hr exposures, each crab was immediately removed from the glass chamber to the respiration chamber. They were then acclimated for three-five hrs in respiration chamber before the measurements. Oxygen consumption was measured using closed system respirometer. An oxygen electrode (1302 Oxygen electrode) was fitted through the lid of the respiration chamber (volume of 750 ml) and connected to the probe holder of oxygen meter (Strathkelvin Instruments oxygen meter model 781). The oxygen electrode was acclimated for 10 minutes before recording $Po_2$ at one hour. Measurements of oxygen consumption were performed in ten individual crab in 50, 75, 100 and 125%SW after 96 hr exposure to 0, 0.75, 1.5, 3 and 6 ppm copper. Refrigerated circulating water bath was used for controlling the temperature (25 ºC) within the respiration chamber.

Weight specific oxygen consumption of animal typically decrease with increasing body weight according to the allometric relation $Mo_2 = aW^b$ $\mu mol.g^{-1}h^{-1}$. Where $a$ is the metabolic rate for an animal of unit mass (1 g in this case), $W$ is the body weight (g) and $b$ is the weight exponent (usually negative)

Weight specific oxygen consumption rates were plotted against body weight and the values of $a$ and $b$ in the above equation were obtained by double logarithmic regression. Differences in mean $Mo_2$ between treatment groups were tested by analysis of covariance and including weight as a covariate. Pairwise comparisons among treatment were made using Scheffe test.

Differences in mean weight specific oxygen consumption rate among groups of crabs would be indicated by differences in the value of $a$, the estimated weight specific oxygen consumption rate for a one gram crab. As, this weight falls outside the range for crabs used in this study, a more meaningful statistical comparison was obtained from estimates of the mean metabolic rate of 17 g crabs which was close to the mean weight of all treatment groups. Thus, individual weight specific metabolic rates were scaled to 17 g weight using the individual weight exponents ($b$) for each treatment group determined as above

$$Mo_{2(17)} = Mo_{2(W)} \times \frac{17^b}{W^b} \quad \mu mol.g^{-1}.h^{-1}$$

where $Mo_{2(17)}$ is the scaled value, $Mo_{2(W)}$ is the measured value and $W$ is the weight of the crab (g).

**Recording of heart rates:** Heart rates were recorded by means of an impedance technique (Hoggarth and Trueman, 1967). Two fine silver wire electrodes were inserted through holes drilled on either side of the heart and held firmly in place with cyanoacrylate glue and rubber dam. These wires were then connected to an impedance coupler and the signals displayed and recorded through a recorder (Kipp and Zonen Flatbed Recorders model BD 112).

Measurements of heart rates were obtained in male blue crabs. After implanting electrode, crabs were left overnight to recover in a chamber with well aerated seawater at 25°C. The sides of the chamber were covered with black plastic to minimize visual disturbance to the animals. Recording wires were of sufficient length to allow the crab freedom of movement within the chamber. Heart rates were monitored onto the paper output of a physiograph pen recorder for periods of 15-20 minutes. Heart rates were measured in 50, 75, 100 and 125%SW after 96 hr exposure to 0, 0.75, 1.5, 3 and 6 ppm copper. The measurements were recorded in five individuals crab per treatment.

Differences in mean heart rate between treatment groups were tested by one-way analysis of variance. Subsequent multiple comparisons of means were performed using the Scheffe pairwise comparisons method. Statistical significance was accepted at $P < 0.05$.

## Results and Discussion

In crustacean, the important surface of the gill tissue (Malins and Ostrander, 1994; Rtal *et al*., 1996) constitutes a main avenue for exchange with the environmental medium. It can thus be assumed that this tissue is one of the first which may have to suffer from toxicity of heavy metals (Malins and Ostrander, 1994). In crabs, *Carcinus maenas*, the gill damaged by copper results in reduce oxygen consumption (Spicer and Weber, 1992) and disrupt cardiac and respiratory activity (Depledge, 1984).

In this work, the effect of 96 hr exposure to copper concentrations on respiration was investigated in *P. pelagicus.* Weight specific oxygen consumption of crabs exposed to different salinities and copper concentration are shown in Fig. 1. $Mo_2$ of individual crabs are plotted against their live mass as regression lines. The oxygen consumption per unit body weight tended to decrease as the body weight increased. The regression equations of the lines and weight specific oxygen consumption of 17 g crabs are presented in Table 1. The results

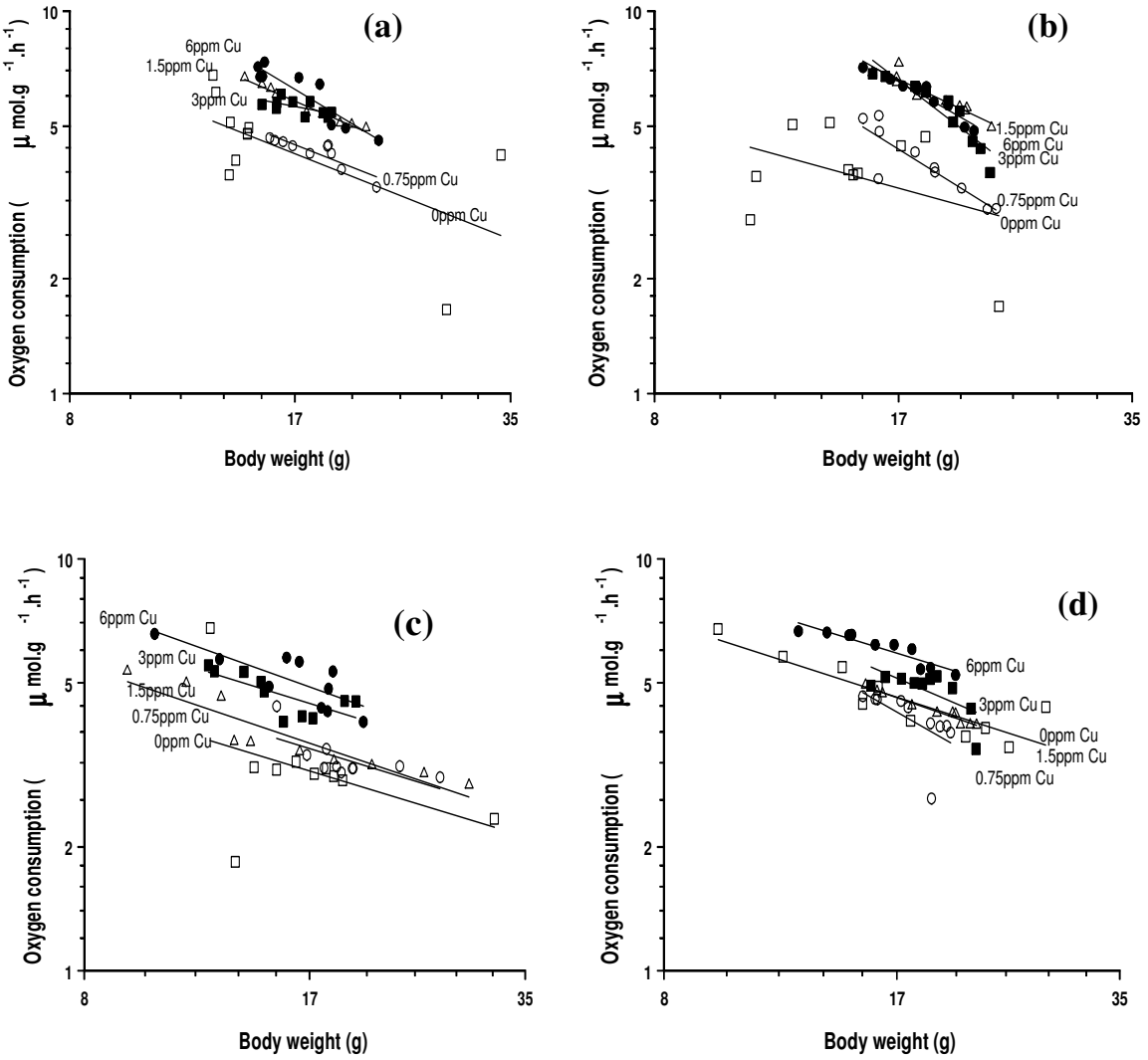


**Fig. 1:** Relationships between weight specific oxygen consumption and body weight of *P. pelagicus* after exposure to different concentrations of copper for 96 hr, temperature 25 ºC, measurements were done in **(**a**)** 50%SW, (b) 75%SW, (c) 100%SW and (d) 125%SW, (copper concentration : 0 ppm ---- , 0.75 ppm ----O, 1.5 ppm ----△, 3 ppm ----■, 6 ppm ----●).

showed weight specific oxygen consumption of *P. pelagicus* increased with decreasing salinities and increasing copper concentration (compared with control: in 100% SW exposed to 0 ppm copper). For 125%SW, the rate is also higher than the control (Fig. 2). Differences in mean $Mo_2$ of the regression line between treatment groups were tested by analysis of covariance including weight as a covariate. The statistic showed weight specific oxygen consumption of *P. pelagicus* after 96 hr exposure to 6 ppm copper in all salinities were significantly different ($P$<0.05) from 0 ppm copper.

Weight specific oxygen consumption increased with the exposed copper concentration. This is consistent with results found for other crustaceans exposed to copper (Bamber and Depledge, 1997; Vosloo *et al*., 2002). The acute toxic action of copper appears to have direct effect mainly toward the gill epithelium, leading to important cytological damage to gill tissue (Nonnott *et al*., 1993; Soegianto *et al*., 1999). Generally, several decapod crustaceans have so called "escape response" in reaction to heavy metal pollution. In an attempt to move away from pollution, the animal's oxygen consumption rate increases from pre-exposure values to support this additional activity (Vosloo *et al*., 2002).

Copper exposure was found to affect cardiac activity in *P. pelagicus* in the present study. The heart rates increased with decreasing salinities and increasing copper concentration (compared with control: in 100%SW exposed to 0 ppm copper). For 125%SW, the heart rate is higher than the control (Fig. 3). When crabs were exposed to copper at concentration of 0 ppm to 6 ppm in 100%SW, heart rates were changed from 198±15 to 236±11 beats.min$^{-1}$, and in 50, 75 and 125% SW, it changed

from 209±5 to 258±9 beats.min$^{-1}$, 201±4 to 239±9 beats.min$^{-1}$ and 216±4 to 237±11 beats min$^{1}$ respectively (Table 2). The statistic showed heart rates of *P. pelagicus* in 50%SW after 96 hr exposure to 6 ppm copper were significantly different ($P< 0.05$) from 0 ppm copper.

The observed increase in heart rate of crabs following exposure to copper was consistent with the findings of previous workers (Depledge, 1984; Bamber and Depledge, 1997). Aagaard and Depledge (1993) reported an increase in heart rate of *C. maenas* exposed to 1 mg l$^{-1}$ copper. They concluded

**Table – 1:** Regression equations for weight specific oxygen consumption ($Mo_2$) of *P. pelagicus* and mean±SE values of 17 g crabs after exposure to different salinities and copper concentrations for 96 hr, temperature 25$^{O}$C.

| Salinity (%SW) | Cu (ppm) | Regression equation | $Mo_2$ of 17 g crabs (µmol.g$^{-1}$.h$^{-1}$) | r | n |
|---|---|---|---|---|---|
| 50 | 0 | $Mo_2 = 32.15\ W^{-0.72}$ | 4.41±0.40 | 0.6387 | 10 |
| | 0.75 | $Mo_2 = 33.40\ W^{-0.71}$ | 4.48±0.06 | 0.8717 | 10 |
| | 1.5 | $*Mo_2 = 52.97\ W^{-0.78}$ | 5.82±0.04 | 0.9842 | 10 |
| | 3 | $*Mo_2 = 14.96\ W^{-0.34}$ | 5.64±0.06 | 0.6168 | 10 |
| | 6 | $*Mo_2 = 132.22\ W^{-1.08}$ | 6.26±0.13 | 0.9207 | 10 |
| 75 | 0 | $Mo_2 = 15.74\ W^{-0.54}$ | 3.59±0.31 | 0.3854 | 10 |
| | 0.75 | $*Mo_2 = 137.29\ W^{-1.22}$ | 4.39±0.13 | 0.8769 | 10 |
| | 1.5 | $*Mo_2 = 90.76\ W^{-0.92}$ | 6.65±0.09 | 0.9395 | 10 |
| | 3 | $*Mo_2 = 465.12\ W^{-1.50}$ | 6.61±0.16 | 0.9243 | 10 |
| | 6 | $*Mo_2 = 157.24\ W^{-1.12}$ | 6.55±0.07 | 0.9560 | 10 |
| 100 | 0 | $Mo_2 = 13.02\ W^{-0.51}$ | 3.19±0.32 | 0.4097 | 10 |
| | 0.75 | $Mo_2 = 14.96\ W^{-0.52}$ | 3.48±0.09 | 0.6953 | 10 |
| | 1.5 | $Mo_2 = 17.68\ W^{-0.56}$ | 3.59±0.09 | 0.9239 | 10 |
| | 3 | $*Mo_2 = 19.12\ W^{-0.51}$ | 4.47±0.11 | 0.7400 | 10 |
| | 6 | $*Mo_2 = 26.86\ W^{-0.60}$ | 4.91±0.15 | 0.7980 | 10 |
| 125 | 0 | $Mo_2 = 22.31\ W^{-0.56}$ | 4.64±0.15 | 0.8804 | 10 |
| | 0.75 | $*Mo_2 = 71.36\ W^{-0.99}$ | 4.28±0.16 | 0.6091 | 10 |
| | 1.5 | $Mo_2 = 25.14\ W^{-0.60}$ | 4.60±0.02 | 0.9812 | 10 |
| | 3 | $Mo_2 = 42.68\ W^{-0.75}$ | 5.15±0.14 | 0.6705 | 10 |
| | 6 | $*Mo_2 = 26.55\ W^{-0.53}$ | 5.91±0.06 | 0.9273 | 10 |

*Asterisks indicated significantly different from 0 ppm in corresponding salinities

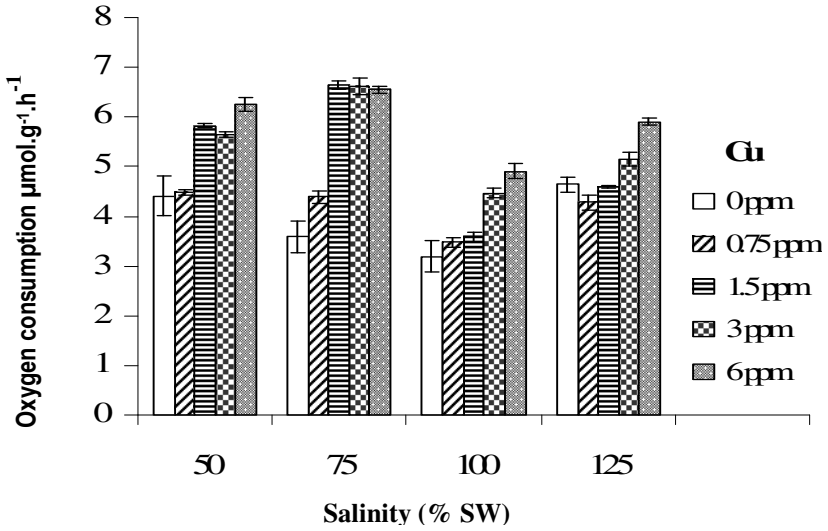


**Fig. 2:** Mean±SE values of weight specific oxygen consumption of 17 g crabs of *P. pelagicus* after exposure to different salinities and copper concentrations for 96 hr, temperature 25$^{O}$C

**Table – 2:** Mean±SE values of heart rates of *P. pelagicus* after exposure to different salinities and copper concentrations for 96 hr, temperature 25°C.

| Salinity | Copper concentrations (ppm) | | | | |
|---|---|---|---|---|---|
| (%SW) | 0 | 0.75 | 1.5 | 3 | 6 |
| 50 | 209±5 | 201±12 | 200±6 | 213±10 | 258±9* |
| 75 | 201±4 | 204±9 | 203±9 | 213±10 | 239±9 |
| 100 | 198±15 | 201±12 | 206±7 | 215±9 | 236±11 |
| 125 | 216±4 | 218±2 | 215±14 | 219±15 | 237±11 |

*Asterisks indicated the significantly different from 0 ppm in corresponding salinities

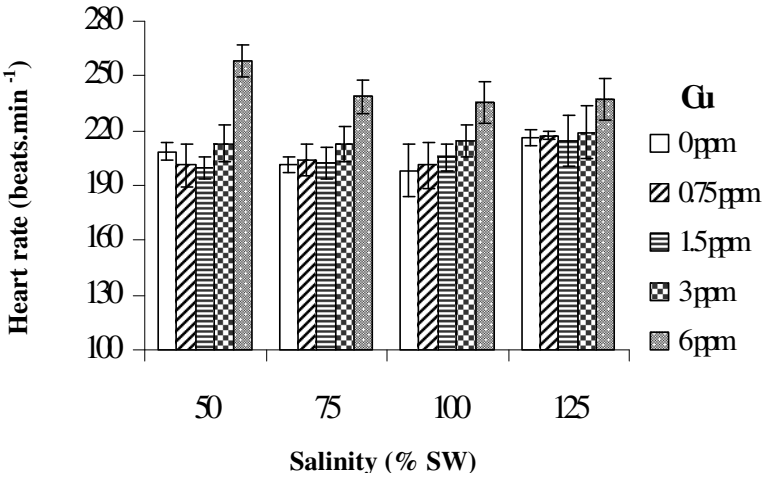


**Fig. 3:** Mean±SE values of heart rates of *P. pelagicus* after exposure to different salinities and copper concentrations for 96 hr, temperature 25 ºC.

that this increase in heart rate may be associated with increased locomotor activity, in addition to, or as opposed to a direct effect of copper on the heart. However, little is known about the direct effect of copper on the mechanisms involved in controlling cardiac activity. Examination of the heart rate of crabs undergoing physical stress gave a similar pattern of response with an increase in rate matching increase in copper concentration. These elevated rates appear to indicate respiratory stress.

Both heart rates and weight specific oxygen consumption increased with increasing copper concentration after a short exposure to 96 hr. However, too long exposure may cause a disrupted in respiratory system by depressed in oxygen consumption (Spicer and Weber, 1992; Harris and Santos, 2000) and suppressed cardiac activity (Depledge, 1984) and exhibited considerable damage to gill. Tissue hypoxia in crabs exposed to copper over several days has been reported by Nonnotte *et al.* (1993), a condition which could readily influence respiratory and cardiac physiology resulting in a greater volume of blood flow demanded per unit of oxygen required for tissue respiration.

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