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Design of an Electronically Operated Flow-Through Respirometer and Its Use to Investigate the Effects of Copper on the Respiration Rate of the Amphipod *Gammarus pulex* (L.)

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The use of oxygen consumption as a measure of metabolism has resulted in the development of many types of respirometer. These can be classified into three types. Firstly, there is the closed system in which oxygen concentration is measured at the beginning and end of the experiment and an organism's respiratory rate calculated from the decrease in oxygen concentration and the volume of the vessel (e.g., Franke 1977). Secondly, there is a system in which respired oxygen is replaced by oxygen from the surrounding air and the resultant change in air volume is measured gasometrically (e.g., Pascoe *et al.* 1968). A third approach, and the one employed in this study, utilises an open flow-through system in which water passes through a chamber containing an animal and the oxygen concentration is measured and compared to that of a reference chamber without an animal. The difference in oxygen concentration is then used to determine the respiration rate of the test animal.

In open flow-through systems excretory products are washed away and water is not left stagnant as may occur in closed techniques. In addition, the open flow technique provides a constant oxygen concentration, avoids stress to animals with oxygen concentration-dependent metabolism and allows the simulation of low oxygen tension environments. Despite these obvious advantages the use of flow-through respirometers has been somewhat limited due to difficulties in calibration and complexity in construction and operation (Edwards and Learner 1960).

The purpose of this investigation was to design a flow-through respirometer which is sufficiently sensitive to detect pollutant-induced respiratory changes in freshwater invertebrates and which permits automated continuous recording of the respiration of several animals maintained individually. In order to evaluate the system the effect of copper (prepared from cupric sulphate, $CuSO_4.5H_2O$) on the respiration of the shrimp *Gammarus pulex* was studied.

MATERIALS AND METHODS

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stoppered at each end with a 6.5 mm Perspex® plug and an 'O'-ring to provide air-tight seals. The chambers are held 2 cm apart, to reduce visual interaction between animals in adjacent chambers (Criddle and Chang 1985), and contained within a large Perspex® cylinder which is fixed to a platform. Each respirometer chamber is individually fed with the relevant aerated toxicant or control solution from vessels filled to the same level as each other (through 0.88 mm diameter vinyl tubing via a Watson & Marlow (Falmouth) multi-channel peristaltic pump model 202U/AA) (Figure 1). Chamber outputs are individually connected to the input valve of a 3-way solenoid (24v D.C., 5 W) with one output port from each solenoid connected together so that solutions flow to waste. The second outputs are connected via 0.88 mm bore tubing to a microprobe holder housing a Strathkelvin (Glasgow) 1302 microcathode oxygen electrode. With the solenoid switched off, solution from a test chamber flows to waste, but when switched on, a solenoid allows solution from the chamber to pass through tubing into the microprobe holder, over the oxygen electrode and then into a waste pipe (Figure 1). Dissolved oxygen concentrations of the water are detected by the oxygen electrode attached to a Strathkelvin meter and electronically recorded. A base-line control chamber of dechlorinated water but without an animal is set up in parallel with the animal test chambers to provide a reference from which an animal's oxygen consumption can be calculated.

A BBC B (Cambridge) microcomputer, electronic interface and driver unit are used to switch the solenoids on and off in the required sequence, to monitor and record to disc the oxygen concentration and to provide the user-interface. A program (BBC BASIC) was developed to achieve these objectives and allow the user to define (i) the number of seconds between recorded oxygen readings (1 sec to infinity), (ii) the duration of the monitoring period for each chamber (2 sec to infinity), (iii) the number of recording occasions, (iv) the acclimation period prior to monitoring and (v) the names of results files.* Design of the respirometer permits the measurement of oxygen uptake by four animals (e.g., three exposed to a pollutant and one control). Since initial activity of test animals is likely to be influenced by the stress of handling during their introduction to the respirometer chamber, it is essential to allow a period of acclimation before respiration is recorded. Propp *et al.* (1982) proposed that the acclimation time should exceed 6x the "system flushing characteristic time" (i.e., chamber volume/solution velocity which for this system is 0.59 hr), and so animals are allowed to acclimate for 4 hr before monitoring. Those factors which may modify the activity of test animals: temperature, photoperiod, light intensity and quality, water flow, animal size, feeding status, etc. are all standardised and monitored.

Each chamber has a 30-min monitoring period (one 'run') before the solenoid switches to the next animal chamber. However, data from the first 12.5 min of every run are discarded because the physical effect of solenoid switching causes an unbalanced period of measurement. Seven such runs are performed on each of the control and three toxicant-exposed animals, constituting more than 4200 water oxygen concentration readings per treatment and sampling period. The oxygen concentration in an animal chamber is subtracted from the concentration in the base-line control chamber and used to calculate the respiration rate (mg 02/g animal dry wt/hr) of each animal:

$$\text{Respiration rate (mg } O_2\text{/g animal dry wt/hr)} = \frac{O_2 \text{ consumed (mg } O_2\text{/L) x flow rate (L/hr)}}{\text{Animal dry weight (g)}}$$

* Both program listing and driver unit circuitry available upon request from author.

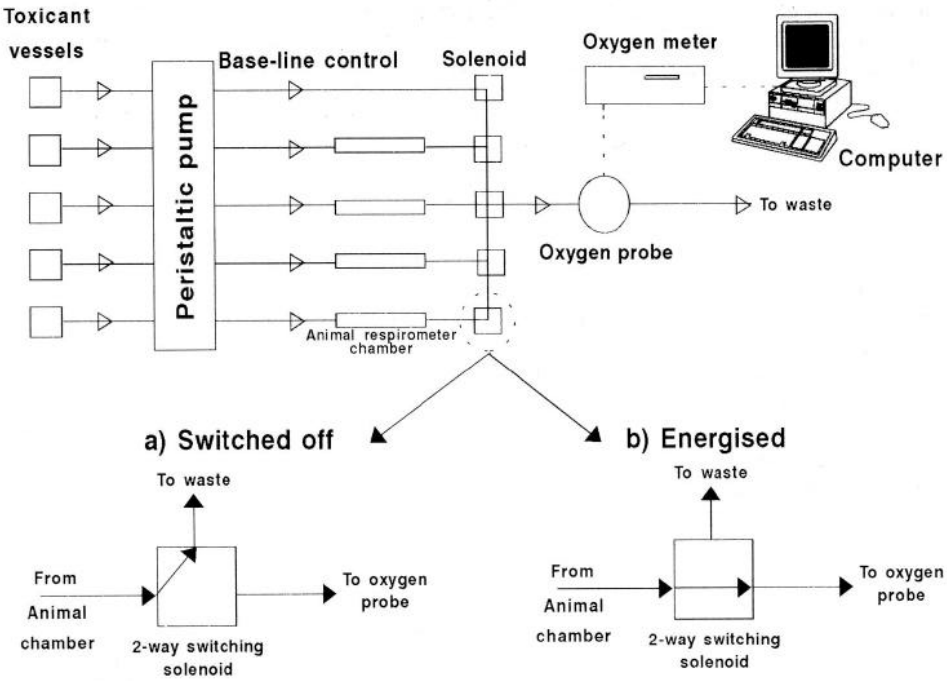

Figure 1. Schematic diagram of respirometry apparatus.

Eighty *Gammarus pulex* (approximately 7 mm in length) were maintained as experimental stock in a 25-L plastic tank containing 3 L of aerated dechlorinated mains water for 4 d prior to the start of the experiment. Animals were fed conditioned (Bird and Kaushik 1985) alder and horsechestnut leaves *ad lib* and maintained under temperature controlled conditions of 13 ± 1°C and photoperiod 16 hr light (McCahon and Pascoe 1988). A computer-controlled, flow-through dosing system was used to provide nominal total dissolved copper concentrations of 10, 20 and 40 µg/L as well as control dilution water. Copper (Cu^{++}) solutions were prepared using cupric sulphate ($CuSO_4.5H_2O$) crystals. These concentrations are below the 48-hr LC50 (47 µg/L) for juvenile *G. pulex* previously determined in this laboratory (Taylor *et al.* 1991). Sixteen animals were randomly selected and individually exposed for 10 d in 3.5-cm diameter pots with nylon net bases (1 mm) suspended in either the copper treatments (4 animals at each concentration) or control water (4 animals). A further 4 animals per treatment and control were exposed on four subsequent days therefore providing 5 batches of 4 animals at each concentration (Table 1).

Animals were provided with excess conditioned leaf material as food in the toxicity test pots but this was removed 24 hr prior to respiration measurements in an attempt to minimise variation in the physiological status of animals (Hayes *et al.* 1992).

One animal from each treatment and control pot was randomly removed after 10 d exposure and its respiration rate determined (7 runs performed) as described above (with toxicant exposure maintained). On each of the subsequent 4 d a further

animal from each treatment and control system was similarly examined. The respiratory rate (mg 02/g animal dry wt/hr) for each of the five animals per concentration was determined as a mean of all seven runs. One-way analysis of variance and Tukey-Kramer multiple comparisons were then performed to identify any significant difference in respiratory rate between treatment and control animals.

Water samples (0.45 pm filtered) were taken every 48 hr from the toxicity test tanks, fixed at the 1% level with ARISTAR® nitric acid and measured against suitable copper standards by furnace atomic absorption spectrophotometry (AAS) on an Instrumentation Model 457. The detection limit for copper was 1.5 ug/L with a relative standard difference of <3.0% and standard operating procedures were followed throughout the analysis. Water quality parameters (pH, conductivity, temperature and dissolved oxygen) were measured every other day with portable meters. Water hardness was determined, by flame atomic absorption spectrophotometry, of the acid-fixed water samples taken for copper analysis.

RESULTS AND DISCUSSION

The measured dissolved total copper concentrations in each of the treatment and control tanks are shown in Table 1.

Table 1. Nominal and measured copper concentrations for each toxicity test.

Nominal copper concentration (µg/L)	Mean measured copper concentration (µg/L) with 95% confidence intervals in parenthesis
Control	5.6 (4.6 - 6.7)
10	10.8 (9.8-11.9)
20	18.5 (17.4-19.5)
40	32.7 (31.6-33.7)

The mean water quality values recorded throughout the test period were pH 8.0 (SE = 0.1), conductivity 286 µS/cm (SE = 5) and total hardness 108 mg/L (SE = 2) as $CaCO_3$. Dissolved oxygen always exceeded 80% of the air saturation value and temperature was 13°C (SE = 1.0).

Animals from all three copper treatments exhibited significantly (one-way ANOVA and Tukey-Kramer test, $P<0.05$) elevated respiratory rates compared with control animals (Figure 2) the lowest observed effect concentration (LOEC) being 10.8 g Cu/L. However, a significant (Tukey-Kramer test, $P<0.05$) reduction in respiratory rate was also observed between the lowest (10.8 µg/L) and highest (32.7 µg/L) copper treatments.

The respirometer facilitated the convenient and repeated measurement of the respiratory rate of *G. pulex*. Similar automated devices have been described (Aagaard *et al.* 1991; Davies *et al.* 1992) and all have the same inherent advantage that animal respiration is measured without disturbing the organism and data are automatically stored during long-term tests by computerised methods. In order to obtain meaningful respiration measurements it is important that the correct acclimation time is employed with respect to animal size, flow rate, and chamber volume. Previous workers have used very different acclimation times, e.g., Naylor

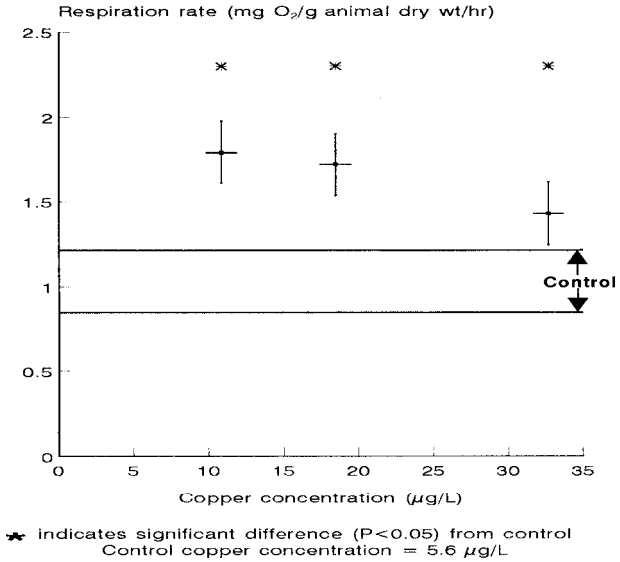

Figure 2. Mean respiration rate (± 95% confidence intervals) of *G. pulex* after 10 d exposure to control water and three copper concentrations.

et al. (1989) used acclimation periods of approximately 15 hr, Wrona and Davies (1984) 6 hr, Davies *et al.* (1992) 1 hr, while Thomas (unpublished data from this laboratory) used just 20 min.

The respiratory rate of control *G. pulex* (mean 1.03 mg O_2/g dry wt animal/hr, SE= 0.093, n = 41) was very similar to that found by previous workers (Nilsson (1974) estimated 1.8 mg O_2/g dry wt/hr; Sutcliffe (1984) 0.83-1.09 mg O_2/g dry wt/hr; Roux and Roux (1969) 1.39 mg O_2/g dry wt/hr) for adult animals under comparable conditions. Application of Sutcliffe's (1984) predictive equation for the mean metabolic rate derived solely from the environmental temperature produced a value of 1.25 mg O_2/g dry wt animal/hr which again compares well to the mean control value quoted above.

Respiration is seen to be a sensitive indicator of copper stress with an LOEC of 10.8 µg/L, a concentration similar to those determined in previous unpublished experiments in this laboratory to affect feeding activity, growth, gut pathology and aspects of metabolism. This suggests that copper has widespread toxicity and interacts with a variety of target systems even at such low copper concentrations. It is interesting to note that Maund *et al.* (1992) determined an LOEC of 14.6 µg Cu/L for changes in the population density of *G. pulex* in a long-term (100-d) study and speculated that the reduced recruitment observed was due to a reduction in the amount of metabolic energy available for reproduction as a result of copper toxicity. It is not clear why, in the current study, respiration decreased at the highest copper concentration compared with the other concentrations. The significant increase in the respiratory rate recorded for *G. pulex* exposed to copper is in agreement with previous workers studying different species. Kapoor and Griffiths (1976) observed a 24-55% increase in oxygen consumption in the stonefly nymph *Phasganophora capitata* exposed to 0.8 to 4.0 mg Cu/L. Similar results have been reported for a marine polychaete, *Nereis virens* (Raymont and Shields 1962) and the sunfish, *Lepomis macrochirus*, in which an increase of 5-60% in respiratory rate was recorded in copper solutions ranging from 0.5 mg/L to 5.0 mg/L (O'Hara 1971). However, Collvin (1984) observed a depression in the

daily maximum respiration rate and growth of perch, *Perca fluviatilis*, on exposure to 87 to 188 µg/L.

The mode of action of heavy metal ions on the invertebrate respiratory system is not well understood. However, it has been reported that accumulation of metals occurs in the gills interferring with the osmoregulatory and respiratory functions of the organ. Exposure to heavy metals eventually culminates in breakdown of gill epithelial cells, and many ultrastructure changes including distension of microvilli and swollen mitochondria (Bubel 1976).

Exposure of *G. pulex* to copper (unpublished data from this laboratory) followed by scanning electron microscopy has revealed changes including lifting of the gill cuticle, an effect which is very likely to impair gaseous and ionic exchange. Doughtie and Rao (1984) also observed lamellar irregularities in the gills of the grass shrimp, *Palaemonetes pugio*, upon exposure to chromium and Rubio *et al.* (1991) in the American red crayfish *Procambarus clarkii* on exposure to lead. Xu (unpublished data from this laboratory) observed disintegration of cell epithelial junctions in the gill of *G. pulex* after 2-d exposure to 1.5 mg Zn/L, although Maltby *et al.* (1990) reported no respiratory changes in *G. pulex* after 6-d exposure to zinc. From a study of the acute toxicity of mixtures of metal cations to *Gammarus lacustris*, De March (1988) suggested that copper and zinc act similarly at acutely lethal concentrations by precipitating, and causing damage to the gill surface, eventually resulting in animal asphyxiation because of the destruction of the gill architecture. In response, the animal might well increase its respiratory rate, as observed in this study, in an attempt to maintain some level of oxygen exchange across the damaged epithelium.

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