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Simultaneous, long-term monitoring of valve and cardiac activity in the blue mussel *Mytilus edulis* exposed to copper

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Abstract Valve and cardiac activity were simultaneously measured in the blue mussel (*Mytilus edulis*) in response to 10 d copper exposure. Valve movements, heart rates and heart-rate variability were obtained non-invasively using a Musselmonitor® (valve activity) and a modified version of the Computer-Aided Physiological Monitoring system (CAPMON; cardiac activity). After 2 d exposure of mussels (4 individuals per treatment group) to a range of dissolved copper concentrations (0 to 12.5 μ*M* as $CuCl_2$) median valve positions (% open) and median heart rates (beats per minute) declined as a function of copper concentration. Heart-rate variability (coefficient of variation for interpulse durations) rose in a concentration-dependent manner. The 48 h EC_{50} values (concentrations of copper causing 50% change) for valve positions, heart rates and heart-rate variability were 2.1, 0.8, and 0.06 μ*M*, respectively. Valve activity was weakly correlated with both heart rate ($r = 0.48 \pm 0.02$) and heart-rate variability ($r = 0.32 \pm 0.06$) for control individuals (0 μ*M* Cu^{2+}). This resulted from a number of short enclosure events that did not coincide with a change in cardiac activity. Exposure of mussels to increasing copper concentrations (≥0.8 μ*M*) progressively reduced the correlation between valve activity and heart rates ($r = 0$ for individuals dosed with ≥6.3 μ*M* Cu^{2+}), while correlations between valve activity and heart-rate variability were unaffected. The poor correlations resulted from periods of valve flapping that were not mimicked by similar fluctuations in heart rate or heart-rate variability. The data suggest that the copper-induced bradycardia observed in mussels is not a consequence of prolonged valve closure.

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Introduction

Blue mussels (*Mytilus edulis*) are able to detect and respond to increased copper concentrations in their environment. Davenport and Manley (1978) measured a threshold detection response of 0.3 μ*M*, which they described as an intermittent movement of the shell valves, along with periodic opening and closing of the exhalent siphon. This behaviour, which they called "testing behaviour" decreased in frequency as the copper concentration increased above the threshold level, until a concentration was reached above which the mussels did not open (3.1 μ*M*).

Numerous researchers have studied the effects of copper on the heart rate of *Mytilus edulis*. Scott and Major (1972) found decreased heart rates in *M. edulis* exposed to 6.3 μ*M* copper for 4 h. Davenport (1977) showed that mussels exposed to 7.8 μ*M* copper for 6 h periods alternated with 6 h periods in clean seawater, exhibited decreased heart rates during the time of exposure. Grace and Gainey (1987) found decreased heart rates in mussels after 4 d constant exposure to copper concentrations ranging between 0.8 and 6.3 μ*M*. The most frequent change noted, apart from a straightforward decrease in rate, was a pattern consisting of a series of beats followed by a pause and then another series of beats (burst activity).

Since mussels can detect environmental copper, and in response close their valves, it is difficult to avoid concluding that the copper-induced bradycardia observed in mussels occurs because they simply isolate themselves from their surroundings. Valve closure leads to a drastic drop in pO_2 and an increase in pCO_2 levels in the mantle cavity-water (Bayne 1971), and it is this

drop in oxygen tension that is believed to attenuate beat frequency.

Although shell adduction may explain the copper-induced bradycardia observed in mussels, no attempts have been made to directly prove this hypothesis. Thus, the aim of the present study was to simultaneously measure valve and cardiac responses in *Mytilus edulis* exposed to copper on a long-term basis in order to establish whether changes in heart rate or heart-rate variability (variations in the instantaneous heart rate) are attributable to valve closure.

Materials and methods

Experimental mussels

Blue mussels, *Mytilus edulis*, (shell length 4.5 to 5.5 cm) were collected from the estuary of the River Exe, Devon, England. Dissolved copper concentrations at this site range between 9 and 53 n*M* (Environment Agency of the UK personal communication). Individuals were transported to the Marine Biological Association, Plymouth, and were maintained in aerated, filtered seawater at 15 to 16 °C and 33‰ S in 20-litre tanks. A constant light regime was employed. The mussels were acclimatised to these conditions for 1 wk prior to experimentation. Water was changes every 3 d. The mussels were not fed during acclimation or during the experimental period.

System overview

A schematic representation of the recording apparatus is shown in Fig. 1. All recordings of valve movements and cardiac activity were obtained non-invasively, with only minimal disturbance to the mussels.

Valve movements

Valve movements were monitored using a Musselmonitor® (Delta consult b.v, Netherlands; De Zwart et al. 1995). Detection of the valve-movement response was based on a high-frequency (HF) electromagnetic induction system. The electronic sensor consisted of two small coils, glued (Loctite super attack®) to opposite shell-halves of the mussel. One coil acted as a transmitter of a magnetic field generated by an HF oscillating current (500 kHz). The other coil, depending on its distance from the transmitting coil, intercepted part of this magnetic field, and a current was induced that was proportional to the distance between the shell-halves (Jenner et al. 1989). To facilitate the undamaged removal of the sensors, Elastoplast® fabric stripping was applied.

The Musselmonitor® was operated using an external power supply (12 V battery) connected through the communications reel. An IBM-compatible PC was connected to the Musselmonitor® via the communications reel. The software program monitoring valve positions within the Musselmonitor® was run in combination with a communications program, PROCOMM+® (Datastorm Technologies, Inc.) within the terminal. The minimum and maximum valve positions were determined for each mussel over a 6 h period prior to experimentation. Minimum valve positions (fully closed) were assessed at the beginning of this period by gently tapping the mussels with a fine glass rod (~5 s) so that they closed their shell halves. Within 10 to 15 min, the mussels would re-open, allowing maximum valve positions (fully open) to be determined over the remainder of the 6 h. Following this evaluation delay, the valve position was expressed as a percentage of the maximum span (fully closed 0%, fully open 100%). Absolute values were recorded also in order to ensure that valve positions had been calculated correctly. One instantaneous value for each individual was sent to the PC at the end of every 5 min. In preliminary experiments, this sampling rate yielded records similar to those obtained using the highest sampling frequency available (1 value every 30 s), whilst minimising the size of the data set. The minimum and maximum valve positions established during the evaluation delay were updated continuously by the Musselmonitor® during the experimental period.

Cardiac activity

Cardiac activity was monitored using the computer-aided physiological monitoring system (CAPMON) developed by Depledge and Andersen (1990). A reflective optocoupler was glued (Loctite super attack®) directly to the mussel shell, on the mid-dorsal line just behind the posterior termination of the hinge. The optocoupler consisted of an infrared light-emitting diode (LED) and a photo-

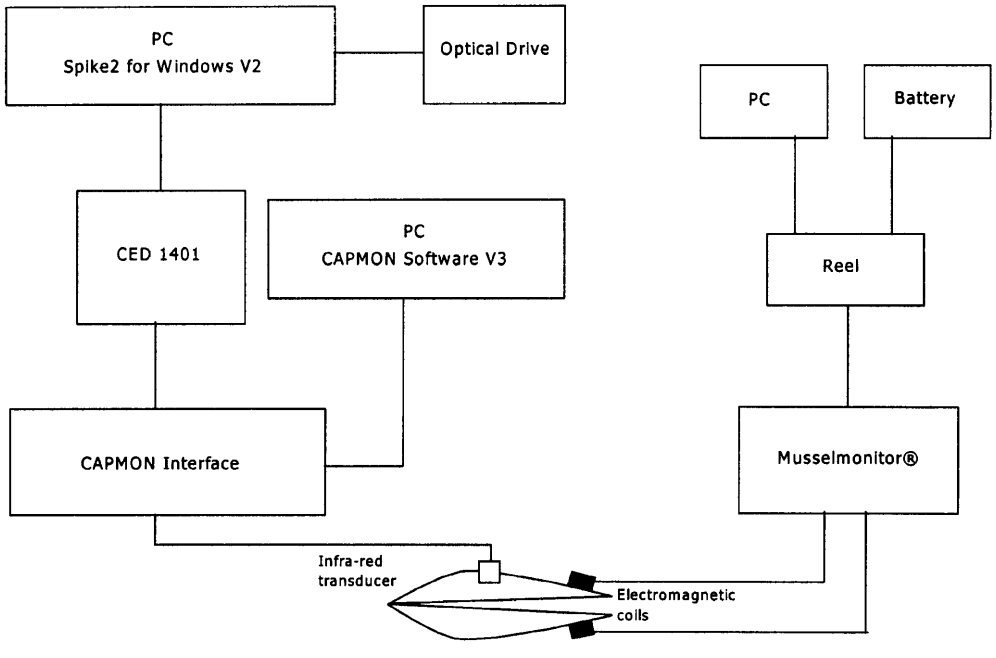

Fig. 1 Overview of recording apparatus

transistor detector. Both elements were mounted parallel to each other and facing in the same direction. Infrared light penetrated the mussel shell to illuminate the heart. The phototransistor detected variations in infrared light intensity associated with the heartbeat, and generated a light-dependent current. This current, which is a function of the reflected light, was then amplified and filtered prior to input to the CAPMON interface. The optocoupler was connected via a fine (~1 mm diam) flexible cable to the CAPMON system, so that the mussel was virtually unrestrained within its aquarium. Cardiac activity was recorded using two separate software packages.

Package 1. The electrical signal from the CAPMON interface was linked to an IBM-compatible PC (via the centronic port) running the standard CAPMON software (Version 3.0). This programme allowed variations in reflected infrared light intensity (associated with the heartbeats) to be viewed as if these were analogue input signals to an oscilloscope. Two adjustable horizontal lines on the screen could then be set to enable triggering of the counting mechanism. When the heartbeat trace passed through both upper and lower trigger levels, the beat was counted. The heart rate was then saved on disk (as beats per minute) for offline analysis. The time resolution of the recordings was then reduced to one data point every 5 min, calculated as the mean number of beats per minute for each 5 min period.

Package 2. The standard CAPMON software described above is limited in providing heart-rate data only. Thus, extra output from the CAPMON interface was required to measure heart rate variability (HRV). To assess HRV, the individual cardiac signals were transferred from the amplified output side of the CAPMON circuitry (between U1D & U2A, see circuit diagram in Depledge and Andersen 1990), and were connected through to a CED 1401plus laboratory interface (Cambridge Electronic Design, UK). The CED 1401plus interface was linked to an IBM-compatible PC running Spike2 for Windows Version 2.0 (Cambridge Electronic Design, UK). Using this software, the heartbeat traces were recorded continuously and saved on optical disk. The heartbeat traces were sampled at a frequency of 50 Hz. The macro-script facility within Spike2 was used to automate the offline analysis of interpulse variability. A modified version of the original Spike2 script automeas.s2 s was used to determine interpulse durations (using peak search-mode). Heart-rate variability (HRV) was expressed as the coefficient of variation (CV) for each 5 min time block.

Experimental protocol

Four mussels were moved to individual 2-litre black aquarium tanks 24 h before experimentation. Sensors were affixed 2 h prior to monitoring (i.e. 2 h, before the evaluation delay for valve movements). This ensured that the mussels had recovered from the sensor attachment procedure before recordings were initiated (Depledge et al. 1996). The mussels were free to move throughout the tanks. Baseline recordings of valve movements and cardiac activity were established for each individual over a 2 d period. Groups of four mussels were then exposed individually to 0, 0.08, 0.8, 3.1, 6.3 or 12.5 μM Cu^{2+} as $CuCl_2$ (equivalent to 0, 0.005, 0.05, 0.2, 0.4 and 0.8 parts per million). A 15.6 μM stock solution was prepared using filtered seawater, and the appropriate working concentrations were added directly to the aquaria. Control mussels (0 μM Cu^{2+}) received 1 ml filtered seawater. Care was taken not to disturb the mussels when the copper was added. Recordings were continued for a further 10 d or until death occurred. Water was changed for each concentration every 2 d.

Data handling and statistics

Neither the valve position data, heart-rate data nor the coefficient of variation data were normally distributed. In fact, these data were bimodal due to periods of valve closure paralleled by complete suppression of the heartbeat. Thus, median values were used as a more robust measure of location than the mean. Median valve positions, heart rates and CVs for each mussel prior to and for 2 d following copper exposure were calculated, and these medians were used to compute percentage change for each individual (median values were not calculated for the full 10 d exposure period because at high copper concentrations some of the mussels died within 3 d). Regression lines of probit percentage-decrease in valve position, inhibition of heart rate and increase in CV versus log $[Cu^{2+}]$ were calculated. This allowed the 48 h EC_{50} (concentration causing a 50% change) to be determined from the regression line for each variable. The Kolmogorov–Smirnov two-sample test was used to examine differences between valve positions, heart rates and CVs before and after copper exposure for each individual.

The product-moment correlation coefficient was used to examine relationships between heart rate and HRV versus valve activity. Significance tests for the correlation coefficients were not appropriate because none of the data was normally distributed (Sokal and Rohlf 1995).

Results

Valve activity

The median valve position of the control *Mytilus edulis* over a 10 d period, was 78% valve open. An example of the characteristic valve movement response of mussels in clean seawater is presented in Fig. 2A. It is clear that the mussel stays open for most of the time and closes only for short periods (5 to 10 min). Occasionally, however, the shells may remain closed for up to 3 h. The time between these long enclosure events may vary substantially between mussels. Addition of ≥0.8 μM Cu^{2+} resulted in a rapid (every 5 to 10 min) opening and closing of the shell valves (“flapping” activity: Fig. 2B). During exposure to high copper concentrations (≥3.1 μM), this behaviour was preceded by a short period (2 to 3 h) of complete valve closure (Fig 2C).

After 2 d exposure to copper concentrations ranging from 0.08 to 12.5 μM, median valve positions exhibited a double exponential decline as a function of the copper concentration (Fig. 3). The average percentage decrease of the valve positions of mussels exposed to the highest concentration of copper was 80%; for the valve positions of mussels exposed to the lowest concentration (0.08 μM) it was 2%. The copper concentration causing a 50% reduction in valve position (48 h EC_{50}) was 2.1 μM.

Typical frequency distributions of valve positions recorded for *Mytilus edulis* 2 d prior to and 2 d following copper exposure are presented in Fig. 4. Preceding copper exposure, the mussels remained open for most of the time, with most valve positions ranging between 50 and 95%. The distribution of valve positions was not significantly different after exposure of mussels to copper concentrations < 0.8 μM ($p > 0.05$) (Fig. 4A). Exposure of individuals to copper concentrations ≥0.8 μM caused a significant decrease in the valve positions ($p < 0.01$), displacing the location of the frequency distributions towards the y-axis (Fig. 4B, C).

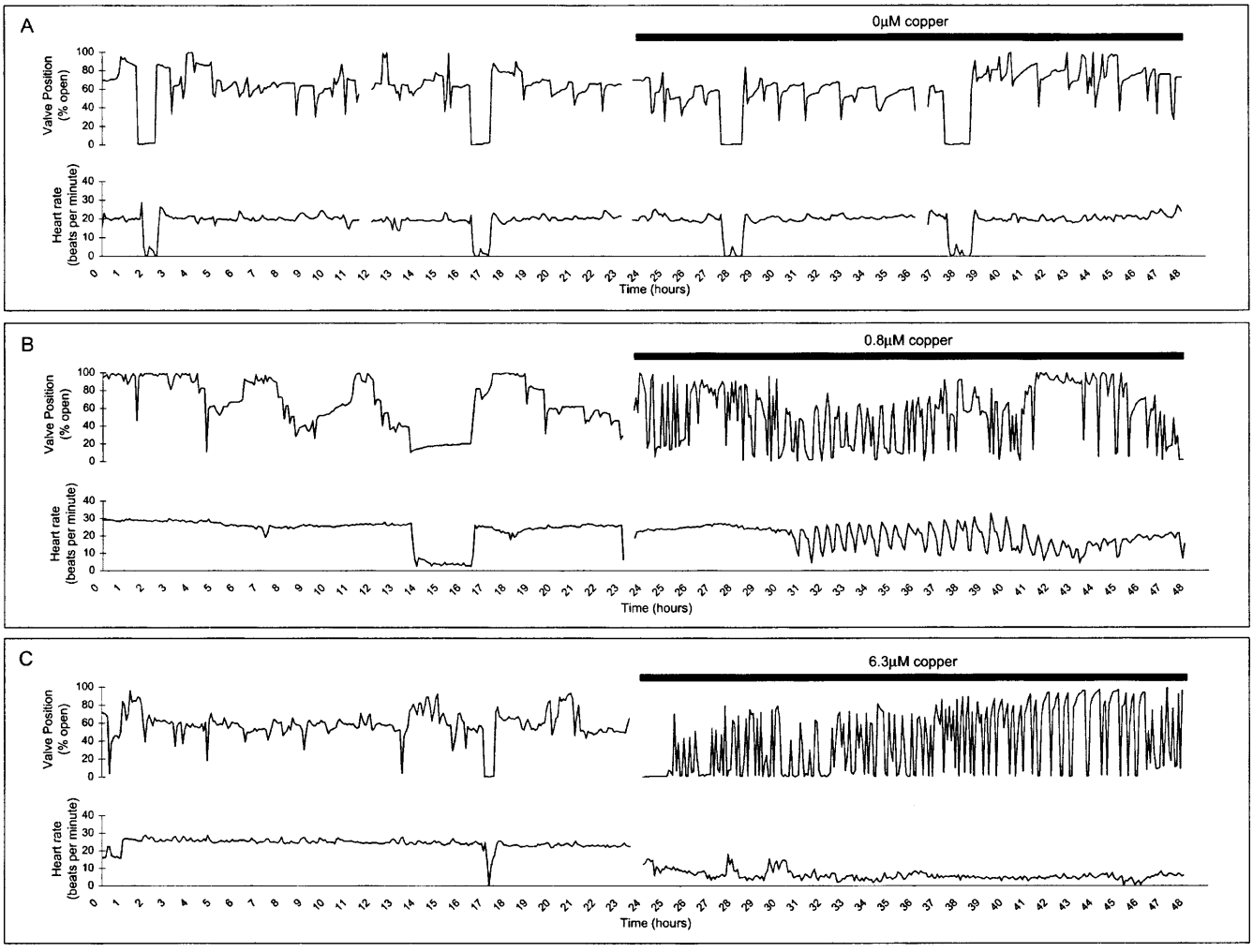

Fig. 2 *Mytilus edulis*. Simultaneous recordings of valve movements and heart rates prior to (0 to 24 h) and following (24 to 48 h) exposure to range of copper concentrations

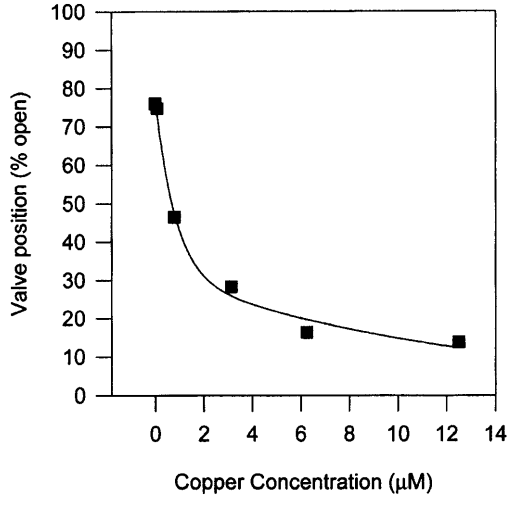

Fig. 3 *Mytilus edulis*. Effect of copper on valve position. Each data point represents median valve position for pooled group of 4 mussels. Regression equation: $f(x) = 45.7\,e^{-1.2x} + 31.5\,e^{-0.08x}$

Heart rate

The median heart rate of control mussels over a 10 d period was 17 beats per minute (bpm). The heart rates of control individuals were notably more constant than their valve movements. Nevertheless, a complete cessation of the heartbeat often accompanied periods of long-term (up to 3 h) valve closure (Fig. 2A). Fig. 5 shows representative tracings of heart rates for mussels exposed to each of the copper concentrations. The heart rates of mussels exposed to 0 and 0.08 μ*M* copper did not appear to change significantly over the 10 d period (Fig. 2A; Fig. 5A, B). Nevertheless, in some individuals, visual analysis of the tracings proved misleading. Mussels exposed to ≥0.8 μ*M* copper showed immediate and long-term changes in heart rate. The immediate response of the mussels to the addition of copper was a protraction of the heartbeats (Fig. 5C–F), causing the heart rate to fall (Fig. 2B, C). Long-term reductions in the heart rate were characterised by a short series of beats followed by a pause (burst activity). Long-term changes in the heart rate were apparent after 5 d exposure to 0.8 μ*M* Cu^{2+} and only 2 h exposure to 12.5 μ*M* Cu^{2+} (Fig. 5C, F).

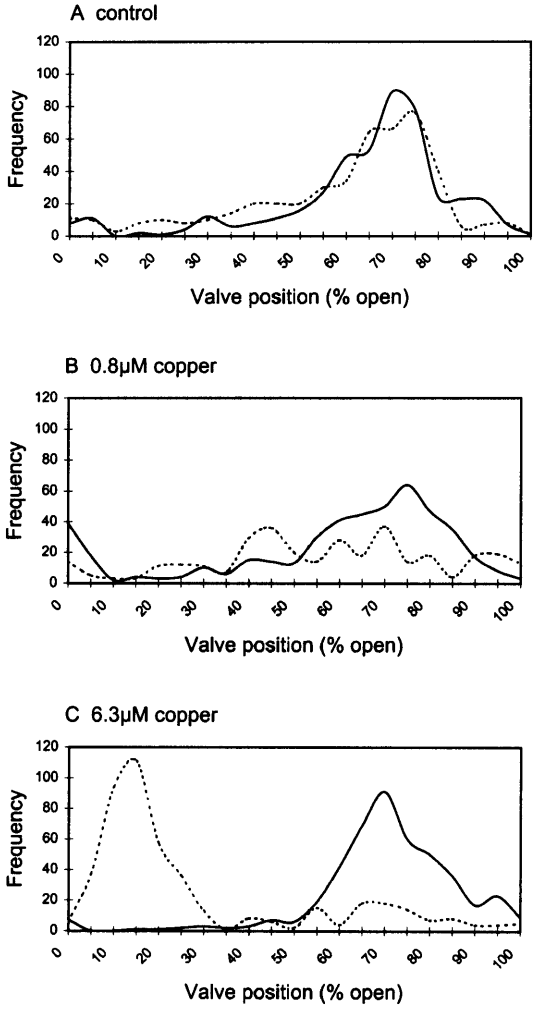

Fig. 4 *Mytilus edulis*. Typical frequency distributions of valve positions recorded 2 d prior to (*continuous lines*) and 2 d following (*dotted lines*) exposure to increasing doses of copper

After 2 d exposure to copper, median heart rates showed a double exponential decay as a function of copper concentration (Fig. 6). The average percentage inhibition of the heart rates of mussels exposed to the highest concentration of copper was 83%; for the heart rates of mussels exposed to the lowest concentration (0.08 μM) it was 17%. The concentration of copper causing a 50% reduction in heart rate (48 h EC_{50}) was 0.8 μM.

Fig. 7 shows typical frequency distributions of heart rates recorded for mussels 2 d prior to and 2 d following copper exposure. Before copper exposure, the heart rates of mussels were steady, with the majority falling between 16 and 22 bpm. Following exposure, a significant reduction in heart rates was recorded for all mussels ($p < 0.01$), with the exception of 2 control individuals and 2 individuals exposed to 0.08 μM Cu^{2+} ($p > 0.05$). The fall in heart rates after copper exposure is evident in Fig. 7A–C as a shift in the locations of the frequency distributions towards the *y*-axis.

Heart-rate variability

The median coefficient of variation (CV) of control mussels over a 10 d period was 0.08 (ratio of standard deviation of interpulse duration to mean). No discernible changes in the heartbeat rhythm of the mussels exposed to 0 and 0.08 μM Cu^{2+} were obvious from the tracings of cardiac activity (Fig. 5A, B). However, as observed with heart rates, the initial evaluation proved deceptive. Heartbeat patterns became irregular in individuals exposed to ≥0.8 μM Cu^{2+} (Fig. 5C–F). The initial effect of dosing the mussels with ≥0.8 μM Cu^{2+} was an erratic beat pattern associated with protraction of the heartbeats. Long-term irregularities were a consequence of burst activity.

After 2 d exposure to copper, median CVs exhibited a double exponential rise as a function of copper concentration (Fig. 8). The average percentage increase in the CVs of mussels exposed to the highest concentration of copper was 2081%; for the CVs of mussels exposed to the lowest concentration (0.08 μM) it was 49.2%. The concentration of copper causing a 50% increase in the CV was 0.06 μM.

Figure 9 shows representative frequency distributions of CVs recorded for mussels 2 d prior to and 2 d following copper exposure. Before copper exposure, the heartbeat patterns of mussels were regular, with CVs ranging between 0 and 0.3. The distribution of CVs of control mussels were not significantly different after exposure ($p > 0.05$) (Fig. 9A). Exposure of individuals to copper concentrations ≥0.08 μM caused a significant increase in the CVs ($p < 0.01$), moving the location of the frequency distributions towards the right-hand side of the graphs in Fig. 9B, C. Furthermore, the frequency distributions of the CVs became more dispersed following copper exposure, corresponding to the alternated periods of regular and irregular beating associated with burst activity.

Heart rate and heart-rate variability versus valve activity

These experiments have demonstrated a relationship between the heart rate and valve activity of *Mytilus edulis* in clean seawater. Fig. 2A shows that high levels of heart rate are associated with valve gaping, whereas prolonged periods of valve closure are usually accompanied by cardiac arrest. Despite these observations, only a modest correlation was established between valve positions and heart rates of control mussels ($r = 0.48 \pm 0.02$). This paradox appears to result from a multitude of short enclosure events that did not coincide with a change in the heart rate (Fig. 2A).

Exposure of the mussels to increasing copper concentrations (≥0.8 μM) progressively reduced the correlation between valve activity and heart rates. At low copper concentrations (0.8 and 3.1 μM), the fall in the correlation coefficients ($r = 0.4 \pm 0.05$ and

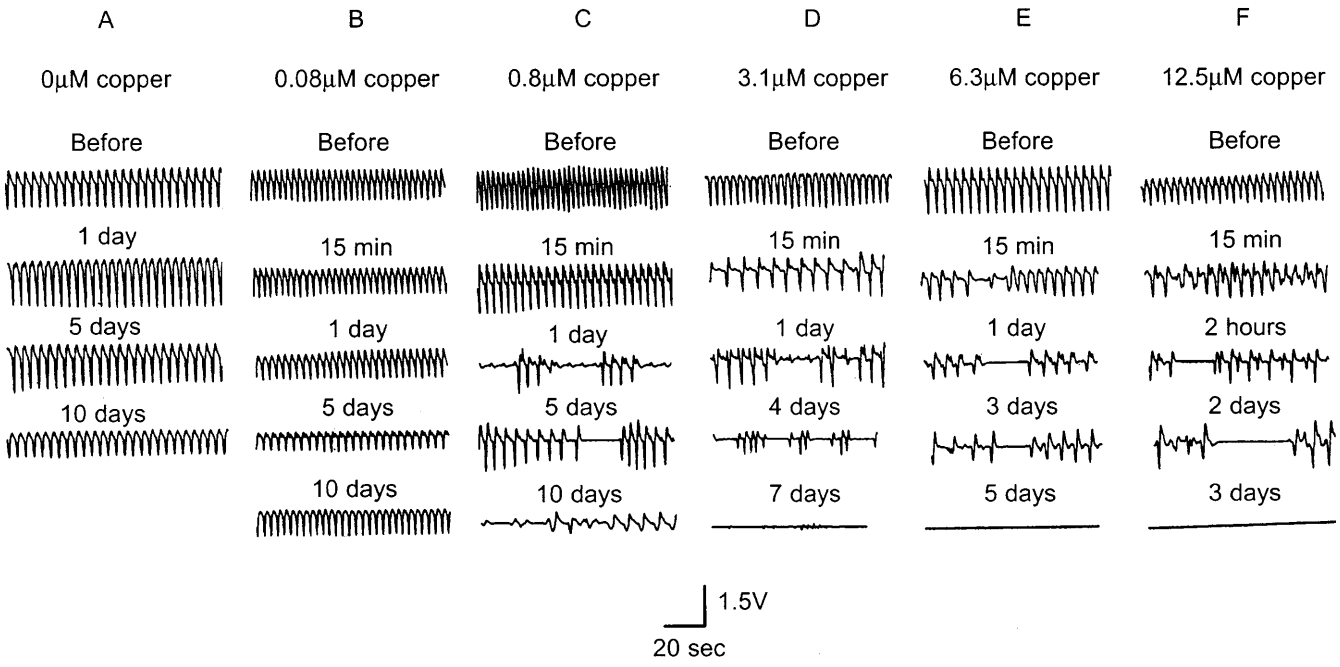

Fig. 5 *Mytilus edulis*. Tracings of heart beats over time for each of the copper concentrations. Visual differences in "before" traces can be explained by positioning of sensors relative to the heart, which varied slightly from individual to individual. The mussels exposed to 12.5 and 6.3 μM copper died within 3 and 5 d, respectively

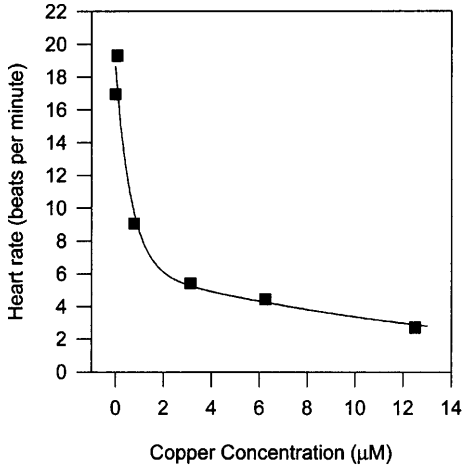

Fig. 6 *Mytilus edulis*. Effect of copper on heart rate. Each data point represents median rate for pooled group of 4 mussels. Regression equation: $f(x) = 12.4\,e^{-1.5x} + 6.3\,e^{-0.06x}$

$r = 0.2 \pm 0.12$) were a consequence of the rapid flapping of the shell valves, a response that was only partially mimicked by fluctuations in the heart rate (Fig. 2B). For mussels dosed with ≥6.3 μ*M* Cu^{2+}, no association between valve activity and heart rate could be observed ($r = 0$). This also resulted from the rapid opening and closing of the shell valves, but for these individuals, heart rates remained relatively constant (between 1 and 5 bpm) (Fig. 2C).

Valve activity was weakly correlated with HRV for control mussels ($r = -0.32 \pm 0.06$). As observed with heart rates, the weak correlation was the outcome of a number of short enclosure events that did not correspond to a change in the heartbeat regularity. Exposure of the mussels to increasing copper concentrations did not affect the correlation coefficients. In this instance, the weak correlation coefficients resulted from periods of valve flapping that were only moderately paralleled by changes in the CVs.

Discussion

The non-invasive valve and cardiac monitoring systems used in the present study on *Mytilus edulis* proved reliable for measuring valve positions, heart rates and heart rate variability (HRV) simultaneously from groups of 4 mussels for 12 d. Furthermore, there was no apparent reason why monitoring could not have been continued for several more weeks or months.

Valve activity

Results from the present experiments indicate that in clean seawater, *Mytilus edulis* keeps its shells open most of the time. Nevertheless, a number of short enclosure events were observed. These rapid phasic adductions probably serve to rid the ctenidia of mucus accumulations, to expel faeces from the rectum through the exhalent siphon, and to expel "pseudofaeces" from the mantle edges (Hiscock 1950).

Mytilus edulis also exhibited periods of long-term valve closure lasting for up to 3 h. A similar phenomenon has been observed in the tropical marine bivalve *Isognomum alatus* (Trueman and Lowe 1971). In common with *I. alatus*, these "rest" periods in *M. edulis*

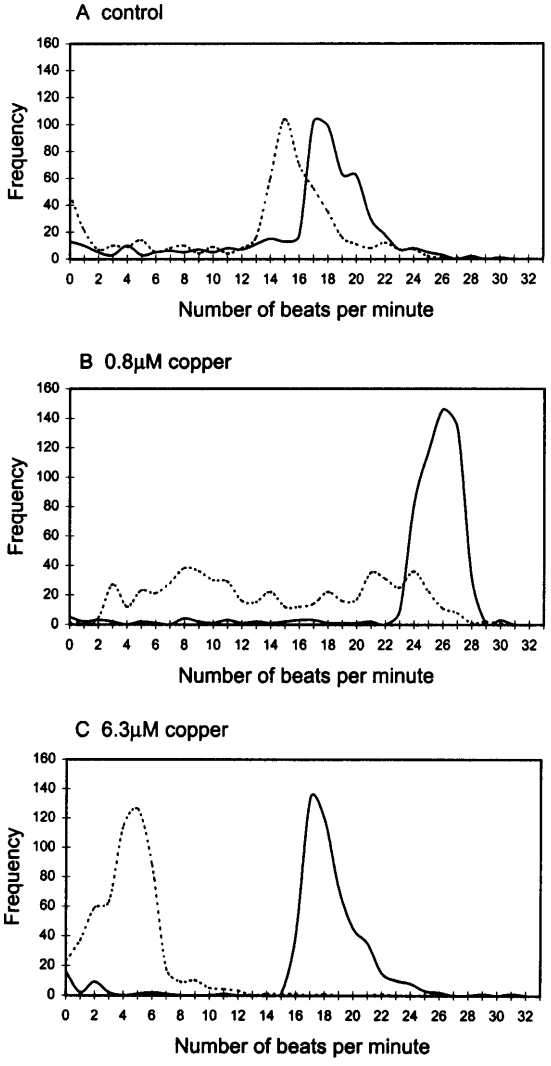

Fig. 7 *Mytilus edulis*. Representative frequency distributions for heart rates recorded 2 d prior to (*continuous lines*) and 2 d following (*dotted lines*) exposure to increasing copper concentrations

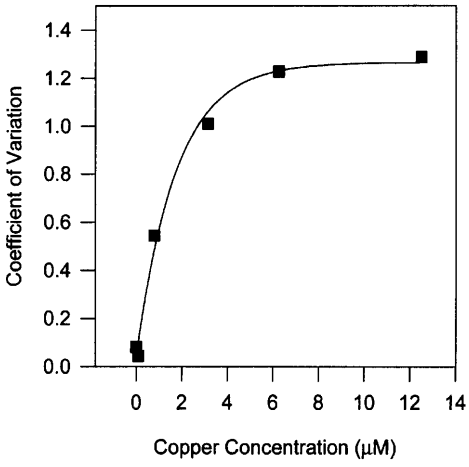

Fig. 8 *Mytilus edulis*. Effect of copper on heart-rate variability. Each data point represents median coefficient of variation for pooled group of 4 mussels. Regression equation: $f(x) = -1.2\,e^{-5.7x} + 1.3\,e^{-1.1-10x}$

appear to be spasmodic rather than rhythmic. In the present study, this lack of rhythmicity probably resulted from the specimens being kept for several days under constant laboratory conditions, since exogenous tidally-correlated patterns of shell opening have been well-documented in this species (Davenport 1981).

The present investigation assessed the long-term effects of copper on the valve movements of *Mytilus edulis*. Initial responses were same as those described by Davenport and Manley (1978), with testing behaviour apparent at a copper concentration of 0.8 μ*M* and complete valve closure at concentrations ≥3.1 μ*M*. Long-term changes (not previously documented) were typified by a flapping of the shell valves resembling "testing behaviour" at all of the copper concentrations tested. Presumably, the flapping behaviour observed during long-term copper exposure refreshes the water in the mantle cavity to relieve any respiratory stress caused by valve closure.

Heart rate

In the present study, the heart rates of *Mytilus edulis* maintained in clean seawater were uniform, except for periods of long-term valve closure. Aerial exposure of *Mytilus edulis* results in partial or complete valve closure, and this is accompanied by bradycardia and occasionally leads to a complete cessation of the heartbeat (Helm and Trueman 1967; Coleman and Trueman 1971). The present study shows that prolonged valve closure during immersion has the same effect on heart rate.

Widdows (1973) demonstrated that starvation gradually reduces the heartbeat frequency of *Mytilus edulis*. Nutritive stress may explain why the heart rates of two control individuals (and possibly two individuals in the 0.08 μ*M* Cu^{2+} group) dropped 2 to 3 bpm below the basal frequency during the course of the present experiments (see, for example, Fig. 7A). The copper-induced bradycardia observed in *M. edulis* in the current study is consistent with earlier publications. However, after 2 d exposure to copper, the value obtained for the copper concentration leading to a 50% reduction in heart rate (0.8 μ*M*) was only approximately one-third of that reported Grace and Gainey (1987) (2.7 μ*M*). This may be due to the use of individuals from different sites. The mussels used in the present study were collected from a reasonably unpolluted site (the 1990–1991 mean copper concentrations in tissues of *M. edulis* from Exmouth = 4.7 μg g^{-1} dry wt: Widdows et al. 1995). In contrast, the mussels collected in Casco Bay, Maine, USA, (as described by Grace and Gainey 1987) are known to contain markedly elevated levels of trace metals, including copper (1990 copper concentrations in tissues of *M. edulis* from Gulf of Maine = 10.3 μg g^{-1} dry wt: O'Connor 1992). Chronic exposure to copper in the environment will induce tolerance to the toxic effects of the metal, and may well decrease the sensitivity of the mussels to experimental exposure (Howell et al. 1984).

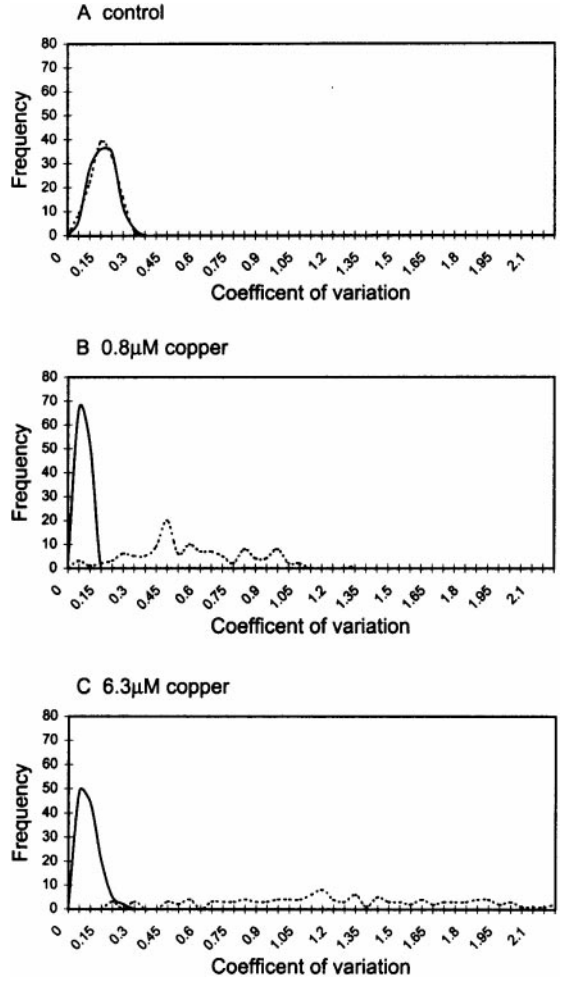

Fig. 9 *Mytilus edulis*. Typical frequency distributions of coefficients of variation recorded 2 d prior (*continuous lines*) to and 2 d following (*dotted lines*) exposure to increasing copper concentrations (5 h of data were analysed at beginning of each day)

Heart-rate variability (HRV)

In clean, well-oxygenated seawater, the beat-to-beat interval of *Mytilus edulis* is extremely regular, confirming earlier observations by Depledge et al. (1996). In contrast, the instantaneous heart rate in mammals is not so constant, but fluctuates around a mean value because of a combination of humoral and neural influences on the pacemaker. The spectrum of the mammalian HRV signal has three distinct frequency components which are attributed to thermal vasomotor activity ($\simeq$0.05 Hz), blood-pressure control-system activity ($\simeq$0.1 Hz) and respiratory arrhythmia ($\simeq$0.25 Hz) (Sayers 1973). The heart-rate power spectrum in the blue mussel contains no such peaks (Curtis 1998). Thus, the absence of marked beat-to-beat variability in *M. edulis* may be because other physiological functions such as ventilation do not exert an influence on the instantaneous heart rate of this species.

Grace and Gainey (1987) reported irregularities in the beat pattern of *Mytilus edulis* following copper exposure. The most frequent change noted was a pattern consisting of a series of beats followed by a pause, then another series of beats (burst activity). In the current study, burst activity was evident within 2 h at the highest concentration of copper (12.5 μM); moreover the time necessary to cause burst activity increased as the copper concentration decreased.

The concentration of copper causing a 50% increase in HRV (0.06 μM) is an order of magnitude lower than the concentration of copper causing a 50% decrease in heart rate (0.8 μM). Furthermore, Depledge et al. (1996) showed that when sensor attachment was mimicked in *Mytilus edulis* (i.e. handling stress), short-lived changes in interpulse duration were detected, but the mean heart rate remained unchanged. These results suggest that HRV in *M. edulis* is a more sensitive indicator of environmental stress than heart rate.

Heart rate and heart-rate variability versus valve activity

Woortman (1926) was the first to demonstrate that heart rate is reduced during periods of long-term valve closure in *Mytilus edulis*. Long-term valve closure and reduced heart rate are not restricted to periods of aerial exposure, since many bivalves, such as *Isognomum alatus*, show spontaneous periods of reduced activity even during periods of continual immersion (Trueman and Lowe 1971). Results from the current study demonstrate that a similar situation occurs in *M. edulis*.

Despite a relationship being established between valve- and cardiac activity in *Mytilus edulis*, cross-correlation between these activities was weak. This was a consequence of a multitude of short-enclosure events that did not correspond to a change in cardiac activity. Thus, it is not generally possible to obtain a measure of valve activity by inspection of heart rate or HRV tracings in *M. edulis*.

Is the disruption of cardiac activity a consequence of valve closure?

Davenport and Redpath (1984) suggested that changes in the cardiac activity of *Mytilus edulis* exposed to copper are the result of valve closure. The experiments here have revealed that this cannot be the only explanation since all test individuals that exhibited changes in cardiac activity did interact with the polluted seawater by frequently opening and closing their shell valves. Notwithstanding this, the possibility does exist that the mussels were still experiencing oxygen deprivation resulting from siphon closure (siphon movements were not measured). For instance, Davenport and Woolmington (1982) showed that *M. edulis* is capable of altering pumping rates, without changing shell gape, by control of the exhalent siphon. Moreover, Manley (1983) showed that closure of the exhalent siphon in *M. edulis* occurs at the same copper concentrations that cause an

initial interruption of the shell valve-movement pattern. Although siphon closure may explain the copper-induced bradycardia observed in this study, it is unlikely, since Scott and Major (1972) showed that, except for the first 90 min exposure, copper concentrations (as $CuCl_2$) below 15.6 μM do not significantly affect the rate of oxygen consumption in *M. edulis* (15 h exposures). Furthermore, they found that concentrations <3.1 μM have no effect even over the first 90 min exposure.

Concluding remarks

The experiments here have revealed that the initial effects of high levels of copper (≥3.1 μM) on *Mytilus edulis* are valve closure and cardiac inhibition. It is probable that a fall in the oxygen tension of the mantle cavity accounts for the disruption of the cardiac activity observed at this time. Presumably, valve closure allows a relatively sedentary bivalve such as *M. edulis* to temporarily avoid contamination and withstand episodic environmental threats. Although such high levels of copper are unusual within the marine environment (30 to 70 nM copper is typical for most estuarine and coastal waters: Davenport and Redpath 1984), concentrations have been known to reach 3 to 7 μM in some severely contaminated coastal waters (see for example Martin et al. 1975).

After 2 to 3 h exposure to high copper concentrations, *Mytilus edulis* flap their shell valves. This behaviour was also observed from the outset in mussels exposed to low levels of copper (0.8 μM). At high copper concentrations, the flapping activity is probably a response to compensate for the oxygen deprivation caused by prolonged valve closure whilst minimising direct contact with the copper containing medium. At low copper concentrations, the rapid opening and closing of the shell valves may reduce the effects of the pollution without incurring the metabolic disadvantages of full isolation. Although flapping activity renews the water in the mantle cavity, mussels still exhibit bradycardia and irregularities in cardiac rhythms. These findings are important because they oppose the earlier view that copper-induced bradycardia in mussels occurs because they simply isolate themselves from their environment. Thus, by excluding valve closure, we are left with the possibility that copper is either directly affecting cardiac muscle contraction or targeting the nervous control system to the heart.

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