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Toxicity of Trace Metals to Juvenile Abalone, *Haliotis rubra* Following Short-Term Exposure

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The value of abalone both commercially and ecologically has increased due a to worldwide population decline. This decline has been attributed to many factors including exploitation by fishing activities, the deterioration of natural habitats and food availability, and possibly pollution of marine environments (Schiel 1993). One particular group of pollutants, the trace metals, are toxic to marine species and can have severe effects on the health of an organism. Each species exhibits unique responses to trace metal exposure. Trace metals are predominantly concentrated in areas surrounding the site of release. such as industrial effluent pipes, sewage outfalls, dumpsites and urban run-off. For the majority of coastal and offshore environments, concentrations of trace metals are commonly below "effect levels" observed in field and laboratory tests (Langston, 1990).

Trace metals include both essential and non-essential metals. Examples of essential metals are Cu and Zn, these metals are vital components of enzymes, respiratory proteins and certain structural elements of organisms (Depledge, 1990). Copper and zinc are the most widely tested trace metals in published literature due to their acute toxicity and bioavailability within marine environments. The mean concentration of Cu and Zn within Port Phillip Bay is $0.47\mu gL^{-1}$, with maximum concentrations reported at $0.63\mu gL^{-1}$ and $1.05\mu gL^{-1}$, respectively (Fabris 1999). The concentration of Cu and Zn in coastal waters in which Haliotis spp. inhabit worldwide range from0.47-76μg Cu/L, and 0.47-3000μg Zn/L (Ferguson, 1983; Tarazona et al. 1991; Fabris et al. 1999; Lee et al, 1996; Apte and Day, 1998; Stauber et al. 2005). Concentrations of Zn in Taiwanese waters, in which abalone are farmed have ranged from 60-300μg Zn/L (Lee, 1996; Lin, 1999)

Non-essential metals with no known biological function include Cd and Hg, which can become incorporated within cellular processes and cause detriment to the cells. Hg and Cadmium are listed as extremely toxic metals on the 'blacklist', and concentrations within coastal waters are maintained at exceptionally low concentrations. The concentration of Hg has been reported at $0.0002\mu gL^{-1}$ in coastal waters (Fabris et al. 1999). The mean concentration of Hg within Port

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Phillip Bay was 0.0017μgL^{-1}, with the maximum concentration of Hg recorded at 0.005μgL^{-1} (Fabris et al. 1999). The concentration of Cd ranges from 0.03-72μg Cd/L in coastal waters (Tarazona et al, 1991; Abdullah and Mustafa 2002; Fabris et al. 1999; Apte and Day, 1998). Port Phillip Bay has a mean concentration of 0.026μgL^{-1}, with a maximum measured Cd concentration of 0.07μgL^{-1} (Fabris et al. 1999).

The effects of acute trace metal exposure to abalone in marine environments have not been extensively investigated. In this study, two essential and two non-essential metals of significance in the marine environment were tested to determine their toxicity to *Haliotis rubra*. A common endpoint employed in toxicity assays is the 96h-LC_{50}, which is the survival rate of 50% of a population following trace metal exposure for 96h. The objective of this study was to determine the 96h-LC_{50} for the trace metals cadmium, copper, mercury and zinc that cause mortality to *Haliotis rubra*. Behaviour of abalone was observed to determine sublethal responses to metal concentrations that did not cause mortality.

MATERIALS AND METHODS

Juvenile *Haliotis rubra* of approximately 1.5yrs of age (shell length 25-35mm) were obtained from Ocean Wave Seafoods (Lara, Australia). These juvenile abalone were transported to the laboratories of the Victorian Marine Science Consortium (Queenscliff, Australia) and acclimated in aerated 100L aquaria (17°C) for 48h prior to commencement of tests.

Static 96hr assays were conducted (ASTM, 1996) to determine 96hr-LC_{50} for each trace metal tested in aerated glass aquaria containing 10L, 1μm filtered seawater with dissolved trace metals. Definitive concentrations for each trace metal were initially determined by performing range finding tests based on a log scale to produce nominal trace metal concentrations. Each concentration and seawater control was tested in triplicate tanks containing ten abalone in each. The trace metals tested were cadmium, copper, mercury and zinc as either chloride or sulphate salts. The concentrations used for each metal were Cd: 1000, 2000, 4000 and 8000μgL^{-1}; Cu: 50, 150, 450, 1350μgL^{-1}; Hg: 40, 120, 360, 1080μgL^{-1}; and Zn: 750, 1500, 3000 and 6000μgL^{-1}. Test solutions were completely renewed at 48h. The trace metal concentration within each test tank was tested by atomic absorption spectrometry (SpectrAA 220 Varian, Australia) at 0h and 48h. Abalone were not fed during the exposure period. Temperature, dissolved oxygen and pH were measured every 24h.

At 24h intervals following the start of exposure, abalone behaviour and mortality was recorded. The behavioural characteristics observed were position of tentacles and their sensitivity to stimuli, surface adhesion by the foot, and the presence of mucus around the gills and in the water column. Results from the separate tests for each trace metal were pooled and percent survival was arcsine-transformed and analysed by single-factor analysis of variance (ANOVA). Median effect (i.e.

LC_{50}) concentrations were calculated using Spearman Karber analysis using the Toxcalc v.5.0© statistical software package (Tidepool®).

RESULTS AND DISCUSSION

There was 100% survival of abalone in control tanks for all trace metals tested. Water parameters remained constant throughout all tests performed (18±1°C; 90±2%O_2; pH 8.06±0.02). There was minimal measured loss of metals from test solution for each concentration over the 48h (Cd = 7%; Cu = 8%; Zn = 5%; Hg = 6%). Within the 96h exposure regime, significant differences in mortality were observed for each trace metal tested. Abalone mortality at 24h, 48h, 72h and 96h increased with exposure time and this was evident for all trace metals tested (Figures 1 - 4).

In the initial stages of exposure, mercury appeared to be the most toxic to *Haliotis rubra* with a calculated 24h-LC_{50} of 335µg/L (95%CI=270-413µg/L). This is compared to copper, which was the second most toxic metal after 24h, with a calculated 24h-LC_{50} of 691µg/L (95%CI=546-875µg/L). Following the 96h exposure, copper was overall the most toxic to juvenile *Haliotis rubra*, with a calculated 96h-LC_{50} of 87µg/L (95%CI=76-98µg/L). The calculated 96h-LC_{50} for mercury was marginally higher at 173µg/L (95%CI=149-201µg/L). Copper and mercury are potentially the most hazardous metals present in the marine environment.

Copper is an essential metal, crucial in normal cellular function at trace concentrations. The toxicity of copper increased over the 96h exposure period, and the health of *Haliotis rubra* declined during exposure. The result determined for exposure of *Haliotis rubra* to copper in this test (96h-LC_{50}=87µg/L) is similar to the calculated 96h-LC_{50} of 50µg/L and 65µg/L reported for *H.cracherodii* and *H.rufescens*, respectively (Martin et al. 1977). Copper has been tested with various marine species and 96h-LC_{50} have range from 370µg/L to 9420µg/L (Okazaki 1976; Kumaragura et al. 1980; Devi 1987; Devi 1997; Karan et al. 1998).

In the initial 48h of exposure, mercury was more toxic to *Haliotis rubra* than copper. As mercury is non-essential for intracellular function, the direct toxic effects to cellular processes may have been experienced in the initial 48h of exposure. The mortality rate of *Haliotis rubra* exposed for the next 48h did not significantly increase between the time intervals of 72h and 96h in contrast to exposure to the essential metal copper. Mercury is toxic not only to *Haliotis rubra* but also to other aquatic species following 96h exposure. Among the trace metals, mercury is considered as one of the most toxic for its high affinity for SH-residues of proteins (Pagliarani et al. 1996). Reported 96h-LC_{50} for marine test species range from 64µg/L to 490µg/L (Ansanullah 1982; Devi 1987; Devi 1997).

In comparison to copper and mercury, *Haliotis rubra* displayed a decreased sensitivity to zinc in the 96h short-term exposure. Zinc produced a 24h-LC_{50} of

4900µg/L (95%CI=4305-5563µg/L). The mortality rate of the abalone increased with time to produce a 96h-LC_{50} of 1730µg/L (95%CI=1524-1971µg/L). Like copper, zinc is essential for normal cellular function at trace concentrations. The essential metals, copper and zinc, have been reported to act pathologically on respiratory systems within an organism (Spicer and Webber 1991). Like copper, zinc is essential for normal cellular function at trace concentrations. Research with molluscs has suggested that the cellular processes of an organism include regulation of excessive zinc concentrations within the body more efficiently than copper concentrations (Anderlini 1974; Young 1975). Though toxic to marine organisms at elevated concentrations, zinc appears to act more slowly than copper, and is much less harmful than copper at equivalent concentrations (D'Silva and Kureishy 1978). The 96h-LC_{50} calculated for *Haliotis rubra* in this test is comparable to the 96h-LC_{50} of 1,200µg/L calculated for the native abalone of Taiwan, *Haliotis diversicolor supertexta* (Liao and Lin 2001). Zinc has been reported to produce 96h-LC_{50} for marine species ranging from 580µg/L to 39,050µg/L (Ansanullah 1976; Devi 1987). The reduced sensitivity displayed in majority of bioindicator species is a function of the regulation of zinc within cellular processes.

For the initial 48h, abalone exposed to cadmium were not significantly affected at concentrations 1000µg/L, 2000µg/L and 4000µg/L with 100% survival in these concentrations. The 24h-LC_{50} and 96h-LC_{50} were calculated to be 6200µg/L (95%CI=5700-6677µg/L) and 3700µg/L (95%CI=3209-4188µg/L), respectively. It may be possible that toxicity of cadmium in *Haliotis rubra* may occur after prolonged exposure greater than 96h. Ansanullah (1976) also reported that test animals were initially unaffected by cadmium, but thereafter, a high proportion died in a short time. Following exposure of various marine species to cadmium, reported 96h-LC_{50} include 2600µg/L to 63000µg/L (Ansanullah 1976; Brown et. al. 1984; Devi 1987; Devi 1997).

The behaviour of juvenile abalone appeared to be affected at sublethal trace metal concentrations. Abalone within control tanks displayed healthy behaviour. This was characterised by each abalone firmly adhering to the tank surface, hastily retracting into the shell when gently prodded, negligible mucus production, and active tentacle movement and response. Observations suggested that after an exposure time of only 24h, abalone exposed to the minimum concentration of each trace metal developed behavioural abnormalities. The rate of behavioural alterations was directly proportional to increasing exposure to metal concentrations.

The amount of mucus produced by the gills was enhanced with increasing metal concentration. Mucus production was evident in the gill area and also in the water column at sublethal concentrations of all trace metals tested following 48h exposure. Mucus production was most evident in *Haliotis rubra* exposed to copper in concentrations as low as 50µg/L. Suffocation may have been a major contributor to the death of *Haliotis rubra* due to the gills secreting considerably

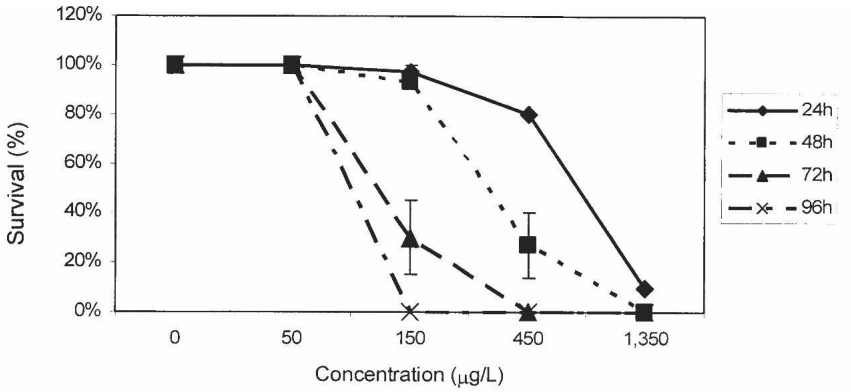

Figure 1. Dose response of abalone (25-35mm) exposed to copper for 96h. Percent survival was measured in each test tank at 24h intervals.

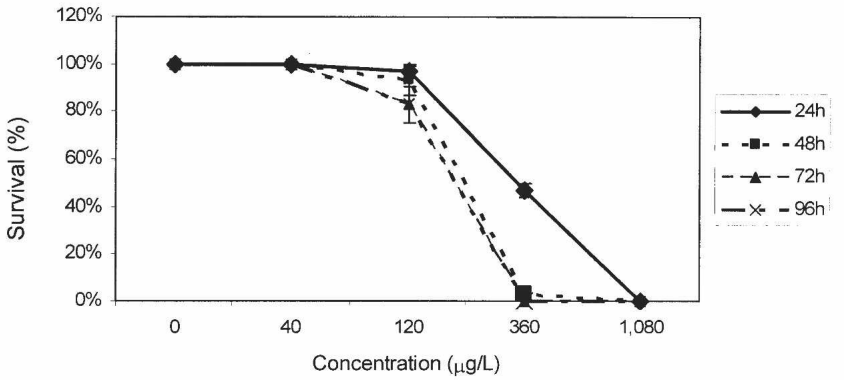

Figure 2. Dose response of abalone (25-35mm) exposed to mercury for 96h. Percent survival was measured in each test tank at 24h intervals.

more mucus than could be excreted into the surrounding water column. This suggestion is supported by the evidence of surplus mucus production by the gills following short-term exposure, possibly inducing the onset of death by asphyxial hypoxia. Trace metal toxicity has been reported to increase the oxygen diffusion distance of the gills of *Haliotis rufescens*, inducing asphyxial hypoxia (Viant et al. 2002). An increase in mucus secretion has been demonstrated as a significant response to heightened trace metal exposure in molluscs (Scott and Major 1972; D'Silva and Kureishy 1978; Sze and Lee 1995; Leung et al. 1999; Yorulmazlar and Gül 2003). Mucus acts as a barrier for the gills, isolating the animal from its environment (Davies and Hawkins 1998). Mucus may protect the gills from trace metal exposure by forming a mucus-metal complex, which is then excreted into the surrounding water column (Martin et al. 1977). Mucus has been reported to be involved in the packing, binding and egestion of faecal material and appears to be an effective agent in depuration of trace metals by the mussels, *Perna viridis* and *Septifer virgatus* (Sze & Lee 1995).

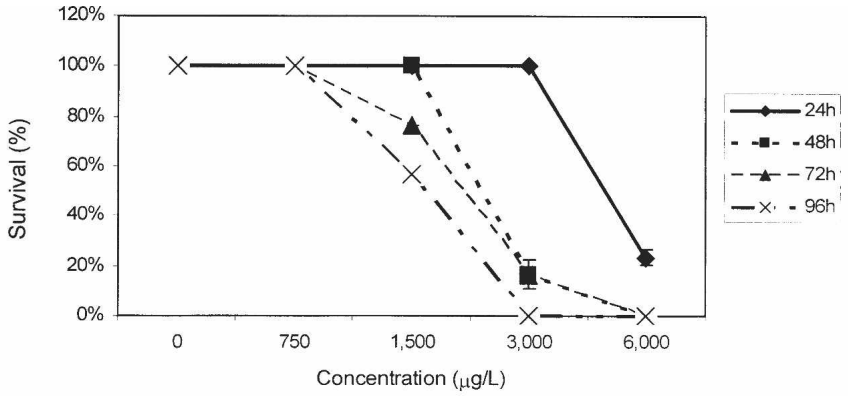

Figure 3. Dose response of abalone (25-35mm) exposed to zinc for 96h.Percent survival was measured in each test tank at 24h intervals.

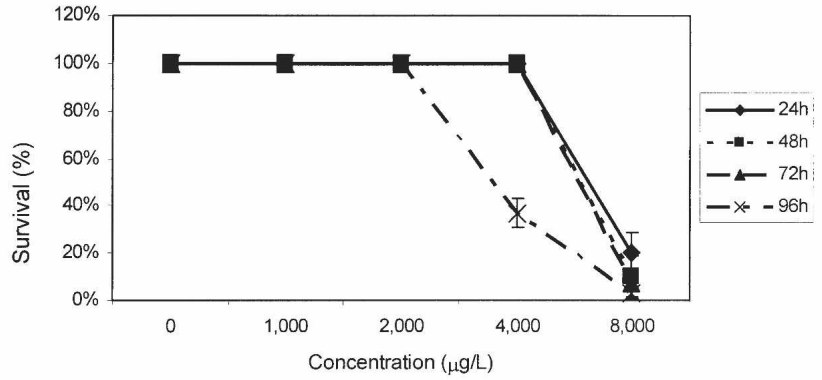

Figure 4. Dose response of abalone (25-35mm) exposed to cadmium for 96h. Percent survival was measured in each test tank at 24h intervals.

Sensory ability of *Haliotis rubra* was severely affected by exposure to trace metals. The reaction time for tentacle retraction and stimulus response was significantly slower compared to abalone in control tanks. In the higher concentrations, *Haliotis rubra* appeared to lose the ability to withdraw tentacles when a gentle stimulus was applied. The capacity for *Haliotis rubra* to adhere to the tank surface was also diminished. In the higher sublethal concentrations, *H.rubra* had lost all ability to hold fast. *Haliotis rubra* that were lacking the ability to adhere had fallen from the side of test tanks, and experienced difficulty or the incapacity to right themselves when positioned abnormally on their shell. This trend was more common in the higher concentrations. *Haliotis rubra* also exhibited the inability to draw their shell close to their foot, exposing the adductor muscle in an abnormal manner.

It may be possible that blood is shunted away from the foot and tentacles to more oxygen-dependent tissues (Donovan et al. 1999). This in turn would affect

cellular metabolic function resulting from insufficient oxygen delivery to the tentacles, adductor muscle and foot of *Haliotis rubra*. It can be assumed that this lack of oxygen delivery to these muscles caused *Haliotis rubra* to experience an inability to adhere to tank surfaces. Similar decrease in muscle function attributed to metal exposure has been observed in the gastropod *Nucella lapillus* (Leung et al. 1999). This lack of oxygen delivery may also increase *Haliotis rubra's* tendency to lift the shell away from the foot and expose the adductor muscle with a decreased ability to pull the shell close to their foot. Normal energy requirements in the foot and sensory organs of the abalone could be reduced in the effort of channelling their energy resource for detoxification and preservation (Leung et al. 1999). Since *Haliotis rubra's* survival is dependent on adherence to rock surfaces and sensory awareness utilising tentacles in natural environments, reduction in muscle function following exposure to trace metals could ultimately prove fatal (Viant et al. 2002).

H.rubra have proven to be as sensitive to short-term trace metal exposure when compared to a variety of other marine species under similar environmental factors. For the majority of coastal and offshore environments, concentrations of trace metals are commonly below "effect levels" observed in field and laboratory tests (Langston 1990). The concentrations deemed detrimental to the survival of abalone in this short-term exposure study are at least an order of magnitude greater than concentrations which are prominent in coastal waters worldwide. It may be possible to include juvenile *Haliotis rubra* as a bioindicator species for the determination of toxicological effects in the marine environment.

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