

EFFECTS OF SALINITY-CYANIDE INTERACTION ON THE MORTALITY OF ABALONE *HALIOTIS VARIA* (HALIOTIDAE: GASTROPODA)

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ABSTRACT

Effects of salinity (25-35 ‰) interaction with cyanide (KCN and NaCN) on survival have been tested on abalone *Haliotis varia*. Highest mortality occurred at a salinity of 25 ‰ and 4 ppm of both forms of cyanide. The lowest salinity could have caused stress, making abalone more sensitive. The effect of cyanide on survival was significant at 1 ppm of KCN and 2 ppm of NaCN ($p < 0.05$), while the salinity and the interaction with cyanide were not ($p > 0.05$). Seemingly, the KCN was more toxic than the NaCN.

INTRODUCTION

Cyanide (CN^-) occurs in marine environments in combination with ions of saline water, forming potassium salt cyanide acid (KCN) and sodium salt cyanide acid (NaCN) compounds. Cyanide also exists as HCN, a very toxic compound. It is naturally found in the marine waters in very low concentrations. However, its concentration may increase due to pollution by cyanide-containing waste.

Cyanide is widely used in industries to extract metals, such as gold from ores, in metal refining, metal cleaning and electroplating operations, and in certain mineral processing operations. Around the Minahasa Peninsular (North Sulawesi, Indonesia) the concentration of cyanide has probably increased due to a contribution from a gold mining company. No specific measurements are available but the company has dumped a hundred ton per day of cyanide containing wastewater to the sea below the tropical thermocline (approximately 82 m depth) through a pipeline (Anonymous 1994). However, the depth of the tropical thermocline is a matter of opinion because no precise data are available.

The objective of the present study is to investigate the effects of salinity-cyanide (KCN & NaCN) interaction on the mortality of the intertidal abalone *Haliotis varia*. Abalones have rarely been used as test organisms for toxicity tests, although they have been used to study the interactive effects of pentachlorophenol and hypoxia (Tjeerdema *et al.* 1991a), the sublethal effects of pentachlorophenol (Tjeerdema *et al.* 1991b), the effects of gas supersaturation on the behaviour, growth and mortality (Leitman 1992), the interactive effects of pentachlorophenol and temperature (Tjeerdema *et al.* 1993), and the effects of salinity and diazinon (Kaligis & Lasut 1997).

MATERIALS AND METHODS

H. varia were collected from January to April 1997 along the Likupang Beach, Minahasa, North Sulawesi. The abalones were kept in the Marine Sciences Laboratory. In accordance with Kaligis & Lasut (1997) they were acclimated for 2 to 5 days at room temperature (24-25 °C). The seawater (salinity 33-34 ‰), was well-aerated and unfiltered. It was changed

every 24 hours. Abalones were fed macroalgae *Gracilaria* sp. during this period. The pH was about 8 when measured before and after the experiment. No food was supplied during experiments.

Abalones with shell lengths ranging from 33.12-50.18 mm were prepared for each experiment conducted in 3-litres plastic bowls, which contained 1 litre of seawater. Three abalones were put in each bowl. The toxicity of KCN and NaCN in four different concentrations (1, 2 and 4 ppm plus one control without cyanide) was tested in relation to 3 salinities (25, 30 and 34 ‰). The cyanide concentrations were based on a preliminary test, which showed that 7.76 and 9.45 ppm were the median lethal concentration (LC₅₀) for 96 hours of both KCN and NaCN. Abalones were scored as being dead according to the criteria by Singhagraiwan *et al.* (1992) and Kaligis & Lasut (1997).

A two-way ANOVA (Sokal & Rohlf 1981; Fowler & Cohen 1990) was applied to analyse if different concentrations of salinity, cyanide and their interaction were affecting the mortality.

RESULTS

No mortality occurred in any of the controls.

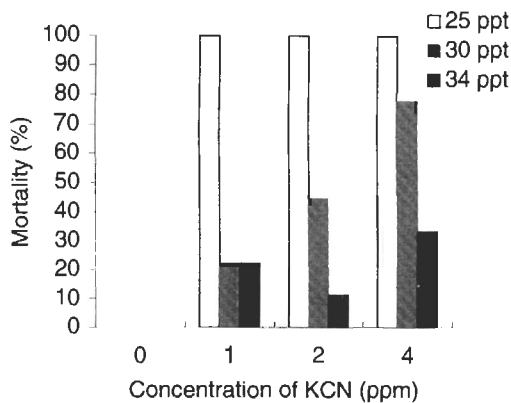


Figure 1. Interactive effect between salinity and cyanide (KCN) on the mortality of the abalone *H. varia* along 96 hours of experiment.

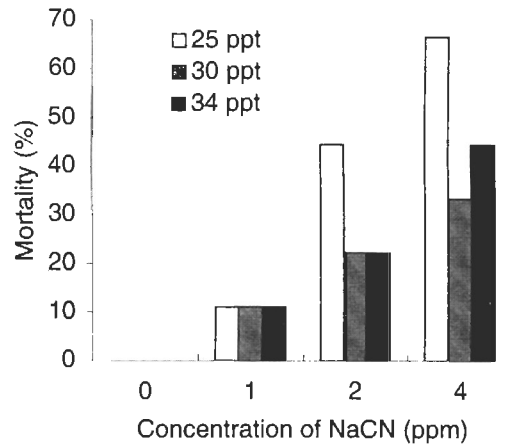


Figure 2. Interactive effect between salinity and cyanide (NaCN) on the mortality of the abalone *H. varia* along 96 hours of experiment.

At a salinity of 25 ‰, 100% mortality was recorded in 1, 2, and 4 ppm (Figs. 1-2). The results of the statistical test (ANOVA) showed that the effects of KCN to the mortality was significant at a concentration of 1 ppm ($p < 0.05$), while the salinity and the interaction between salinity and KCN were not affecting mortality ($p > 0.05$).

In the interactions between 1, 2 and 4 ppm of NaCN and 25 ‰ S, the mortality was 11, 44 and 66 % respectively. In 1, 2, and 4 ppm of NaCN and 34 ‰ S, mortality was 11, 22 and 44 % respectively. The NaCN from a concentration of 2 ppm was significantly affecting the mortality ($p < 0.05$), while the salinity and the interaction between salinity and NaCN were not affecting mortality ($p > 0.05$).

DISCUSSION

Kaligis & Lasut (1997) reported that the combination between salinity and the insecticide diazinon resulted in mortality of the abalone *H. varia*; especially in low salinity (25 ‰). The same trend was found in the present study. Mortality was highest in 25 ‰ and the mortality depended on the concentration of KCN and NaCN (Figs. 1 & 2).

H. varia is thriving in salinities of 32.5 to 35 ‰ (Fuse 1981). The abalone is moderately tolerant to seawater of 25 ‰ S without acclimation and to a salinity of 20 ‰ S with acclimation (Kaligis & Lasut 1997). However, 25 ‰ S is lower than the optimum and may cause stress making the species more sensitive to pollutants.

KCN was relatively more toxic than NaCN. The biochemical action of cyanide is that it deprives the body of oxygen by acting as a chemical asphyxiant. Cyanide inhibits an enzyme involved in the oxidative phosphorylation by which the body utilises oxygen. It has been found that mitochondria deprived of oxygen fail to show significant oxidative phosphorylation (Edwards & Hassall 1980; Manahan 1992). The inhibited enzyme is ferricytochrome oxidase, an iron-containing metalloprotein that acts as a final acceptor of electrons. Cyanide bonds to the iron (III) of the ferricytochrome enzyme, preventing its reduction to iron (II). The result is that ferrous cytochrome oxidase, which is required to react with oxygen, is not formed and utilisation of oxygen in cells is prevented, leading to rapid cessation of metabolic processes (Edwards & Hassall 1980; Manahan 1983; Bohinski 1987; Manahan 1992).

Brachet (1957) reported that cyanide inhibited respiration in developing sea urchin eggs. He, furthermore, stated that many cytological abnormalities were found, such as signs of degeneration of the chromosomes. Lasut & Lintong (1998; unpublished data) found that larvae of sea urchins were malformed if the eggs had been exposed KCN.

Efforts to reduce the toxicity of cyanide in an effluent can be done by alkaline chlorination or by catalytic oxidation. However, cyanide wastewater containing nickel or silver is difficult to treat by alkaline chlorination because of the slow reaction rate of these metal complexes (Lankford 1990).

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REFERENCES

- Anonymous, 1994. Studi analisis dampak lingkungan (*in English*: Environmental impact analyses study). Laporan Utama. Kegiatan Pertambangan Emas di Minahasa dan Bolaang Mongondow, Sulawesi Utara, Indonesia. PT. Newmont Minahasa Raya. pp. 3-7.
- Brachet, J. 1957. Biochemical cytology. Academic Press Inc. - Publishers. New York. 535 pp.
- Bohinski, R.C. 1987. Modern concept in biochemistry. Fifth Edition. Chapter Fifteen: Oxidative phosphorylation. Allyn and Bacon, Inc. Boston. Pp. 567-604.
- Edwards, N. A. & K. A. Hassall. 1980. Biochemistry and physiology of the cell: an introductory text. Second edition. - McGraw-Hill Book Company (UK) Ltd. 448 pp.
- Fuse, D. M. 1981. Notes on the biology of *Haliotis varia* and *H. asinina*. - Fisheries Research Journal of Philippine **6**(1): 39-49.
- Fowler, J. & L. Cohen. 1990. Practical statistics for field biology. - John Wiley & Sons. Chichester. 227 pp.
- Kaligis, F. G. & M. T. Lasut. 1997. Effects of salinity and diazinon on the abalone *Haliotis varia* (Gastropoda: Haliotidae). - Phuket Marine Biological Center Special Publication **17**(1): 115-120.

- Lankford, P. W. 1990. Removal of metals to nontoxic levels. Page 98-124 in P. W. Lankford & W. W. Jr. Eckenfelder (eds.). Toxicity reduction in industrial effluents. - Van Nostrand Reinhold. New York.
- Leitman, A. 1992. The effects of gas supersaturation on the behaviour, growth and mortality of red abalone, *Haliotis rufescens* (Swainson). Page 75-85 in S. A. Shepherd, M. J. Tegner & S. A. Guzmán del Prío (eds.). Abalone of the world: Biology, fisheries and culture. - Fishing News Books, Oxford.
- Manahan, S.E. 1983. Environmental chemistry. Fourth edition. - Willard Grant Press, Boston. pp. 612.
- Manahan, S. E. 1992. Toxicological chemistry. Second edition. - Lewis Publishers. Boca Raton. pp. 449.
- Singhagraiwan, T., M. Doi & M. Sasaki. 1992. Salinity tolerance of juvenile donkey's ear abalone, *Haliotis asinina* Linne. - Thailand Marine Fisheries Research Bulletin **3**: 71-77.
- Sokal, R. R. & F. J. Rohlf. 1981. Biometry. The principles and practice of statistics in biological research. Second Edition. - W. H. Freeman and Company. New York. 859 pp.
- Tjeerdema, R. S., R. J. Kauten & D. G. Crosby. 1991a. Interactive effects of pentachlorophenol and hypoxia in the abalone (*H. rufescens*) as measured by in vivo super (31)P-NMR spectroscopy. - Aquatic Toxicology **21**(3-4): 279-294.
- Tjeerdema, R. S., T. W. Fan & D. G. Crosby. 1991b. Sublethal effects of pentachlorophenol in the abalone (*H. rufescens*) as measured by in vivo (31)P-NMR spectroscopy. - Journal of Biochemistry & Toxicology **6**(1): 45-56.
- Tjeerdema, R. S., R. J. Kauten & D. G. Crosby. 1993. Interactive effects of pentachlorophenol and temperature in the abalone (*H. rufescens*) as measured by in vivo super (31)P-NMR spectroscopy. - Aquatic Toxicology **26**(1-2): 117-132.