EFFECTS OF SALINITY AND DIAZINON ON THE ABALONE *HALIOTIS VARIA* (GASTROPODA: HALIOTIDAE)

F. G. Kaligis & M. T. Lasut

Department of Marine Sciences, Laboratory of Marine Ecology, Faculty of Fisheries, Sam Ratulangi University, Jl. Kampus Bahu, Manado 95115, Indonesia

ABSTRACT

The effects of salinity and diazinon were tested on abalones, *Haliotis varia*, using a bioassay technique. Specifically, three kinds of test were conducted separately: salinity, diazinon, and salinity-diazinon interaction. In the salinity test, the tolerance to salinity was interpreted as survival of the abalone after instantaneous and gradual reduction of the salinity. Abalones survived 100 % in high salinities, 30-35 ‰, but died within 5 days if the salinity was gradually reduced to 15 ‰. In the diazinon test, median lethal concentrations (LC_{50}) for 12, 24, 36, and 48 hours were 10.1, 5.2, 4.8, and 2.3 ppm, respectively. In the salinity-diazinon interaction test, the salinity and their interaction did not show any effects ($P > 0.05$) on the test animals in 48 hours. In all treatments, diazinon in concentrations of 1.2, 2.3 and 3.5 ppm, caused significant mortality ($P < 0.05$) in all salinities tested (34, 25, and 20 ‰). Obviously, the highest mortality occurred in the highest concentration of diazinon and the salinity tested. It was concluded that the salinity indirectly caused the high toxicity of the diazinon.

INTRODUCTION

Diazinon is a member of the group of organophosphorous insecticides (together with parathion). Many studies on the effects of insecticides in marine organisms show that chemicals of that group inactivate the enzyme cholinesterase (ChE) and can break down the neurotransmitter acetylcholine (ACh) in synapses of the nervous system and thereby disrupt the nervous co-ordination. Organophosphorous insecticides increase mortality, inhibit growth and reproduction in marine invertebrates (Connel & Miller 1984: 199; Persoone *et al.* 1985; Rompas *et al.* 1989; Kobayashi *et al.* 1990; Monserrat *et al.* 1991; Rodriguez & Pisano 1993; Lasut 1996), and they are also toxic to humans (Gallo & Lawryk 1991). However, the chemicals are still widely used as biocides to control agriculture pest insects, including foliage and soil insects in Minahasa and Bolaang Mongondow of North Sulawesi Province, Indonesia (Sembel *et al.* 1991).

The abalone *Haliotis varia* Linné, 1758 is common in the intertidal of the North Sulawesi Province, Indonesia (Kaligis 1994). There are strong daily and seasonal fluctuations in water salinity which require physiological adaptations (osmoregulation) of the animals living there. In consequence, we first studied the salinity tolerance of the abalone *H. varia*. Then, the median lethal concentrations (LC_{50}) of diazinon, and finally, the combined effects of salinity and the insecticide on the abalone.

MATERIALS AND METHODS

Abalones, *H. varia*, were collected in June and July 1996 along Teep-Amurang and Likupang beaches, Minahasa, North Sulawesi Province, Indonesia. The test animals were reared in the Laboratory of Marine Ecology, Department of Marine Sciences, Faculty of Fisheries, University of Sam Ratulangi, Manado, Indonesia. The cultivation procedures were modified from Singhagraiwan *et al.* (1992). The animals were acclimated to laboratory conditions for 1 to 5 days in stagnant, aerated and unfiltered sea water, which was changed every 24 hours. All experiments were carried out at a water temperature of 22-23 °C. Solubility of the organophosphorous insecticide Diazinon (0,0-diethyl 0-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) (Gallo & Lawryk 1991) is 40 ppm in water at 20 °C.

Salinity tests
Two kinds of tests were conducted to assess the salinity tolerance of *H. varia*.

Exp. 1: survival after instantaneous reduction of salinity. The experiment was conducted to assess the lethal salinity after a sudden change. Nine animals (shell length 32.8-47.0 mm) were kept in three 3-litre plastic bowls (three animals in each) with 1 litre of water at the salinities of 0, 5, 10, 15, 20, 25, 30, and 35 ‰ during 7 days. The animals were fed with *Gracilaria* sp. (Singhagraiwan *et al.* 1992) collected from their habitats. The water was changed daily with water of the same salinity as the initial one. During this change, the animals were counted and dead individuals removed. The animals were judged as dead according to following criteria:
a) the soft body showed no response when touched by hand (Singhagraiwan *et al.* 1992).
b) the ventral part of the body became pale (pers. obs.).
c) category a and b did not recover after they were returned to clean water.

Exp. 2: survival after gradual reduction of salinity. The effect of acclimation on the lethal salinity level was tested. Animals of 34.5-45.0 mm shell length were placed in the bowls at a salinity of 35 ‰. The salinity was then reduced daily at the rate of 2.5 ‰ until reaching 100 % mortality of test animals. Number of animals, type and shape of the bowls, volume of water, food supply, and counts of dead animals were carried out as described for Exp. 1.

Diazinon and salinity-diazinon interaction tests
The tests were performed according to Abel (1991). Two kinds of experiments were conducted:

Exp. 3: LC_{50}. Short-term tests for the determination of LC_{50} of diazinon were carried out on nine individuals (shell lengths 34.2-43.7 mm). The animals were placed in three 3-litre plastic bowls (three snails in each) with 1-litre of sea water of the optimal salinity, 32.5-35 ‰ (Fuse 1981). Six concentrations of diazinon were tested: 0.06, 0.12, 0.24, 0.48, 0.96, and 1.92 ppm and compared with a control. They were chosen on the basis of the results of pilot tests in order to find the range of critical test concentrations. The water and the bowls were renewed every 24 hours, and dead animals were simultaneously counted (same criteria as in Exp. 1). The animals were not fed during the tests. The tests were terminated after 48 hours and the LC_{50} was calculated.

Exp. 4: salinity-diazinon. The experiment was conducted to study the effects of salinity on the toxicity of diazinon. After the values of median lethal concentration (LC_{50}) of diazinon were obtained in the LC_{50} test above, three concentrations of salinity (20, 25, and 34 ‰) and four concentrations (0, 0.5, 1, and 1.5 the Toxic Unit (TU)) of diazinon were selected to investigate effects of both salinity and diazinon on mortality of the animal. TU = concentration of diazinon/LC_{50} (Rand & Petrocelli 1985; Monserrat *et al.* 1991). Three replicates and different animal shell length (30.5-45.5 mm) were used. Otherwise the number of animals, type and shape of the bowls, volume of water, duration of experiment, and count of dead animals were carried out as in Exp. 3.

Analyses of Data
The median lethal concentrations (LC_{50}) of

diazinon were calculated by using probit analysis according to Finney (1971) combined with the method described by Buhagiar & Abel (1991). Two-way ANOVA (Fowler & Cohen 1990) was applied to test whether the concentrations of diazinon and salinities (diazinon-salinity tests) affected the mortality.

RESULTS

Salinity tests

Fig. 1 shows the survival after instantaneously reduced salinity (Exp. 1). All animals placed in sea water of 0-15 ‰ salinities were dead within one day, while those in the salinity of 20 ‰ could survive for 2 days. The test was terminated after 7 days. During that period survival was 44.4 % in 25 ‰, and 100 % in 30 and 35 ‰ salinities.

Fig. 2 shows the survival after gradual reduction of the salinity (Exp. 2). All animals survived gradual reduction from 35 to 30 ‰. At a gradual reduction to 25 and 20 ‰, the survival was 55.5 and 22.2 % respectively. All the animals died within 5 days if the salinity was gradually reduced to 15 ‰.

LC_{50} for diazinon

The LC_{50} for diazinon after 12, 24, 36, and 48 hours of exposure were 10.1, 5.2, 4.8, and 2.3 ppm respectively (Exp. 3 in sea water of the optimal salinity, 32.5-35 ‰).

Salinity-diazinon interaction test

Fig. 3 shows the effects of salinity on the toxicity of diazinon during 48 hours of exposure. Increase of the concentrations of both diazinon and salinity are followed by increased mortality of the animals. Diazinon in low concentration, even in optimum salinity (34 ‰), caused mortality of the animals. No mortality was found in the control (0 ppm of diazinon) at the salinities tested (25, 30 and 34 ‰). The highest mortality occurred at 3.48 ppm diazinon and 25 ‰ salinity. The salinity and its interaction with diazinon did not show any significant effects ($P>0.05$). Diazinon in concentrations of 1.2, 2.3, and 3.5 ppm, caused significant mortality ($P<0.05$) in all the salinities tested (Exp. 4).

DISCUSSION

Salinity tolerances

H. varia is a marine species thriving in salinities of 32.5 to 35 ‰ (Fuse 1981). The snail is moderately tolerant to a salinity of 25 ‰ without acclimation and to a salinity of 20 ‰ with acclimation (tolerance estimated after 5 days), indicating a differences in sensitivity dependant upon the way salinity is reduced. Instantaneous reduction

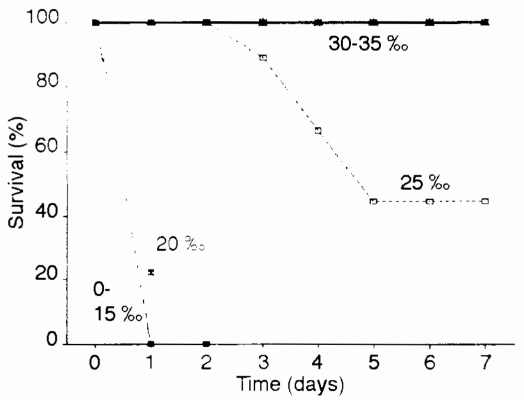

Figure 1. Survival after instantaneously reduced salinity (Exp. 1).

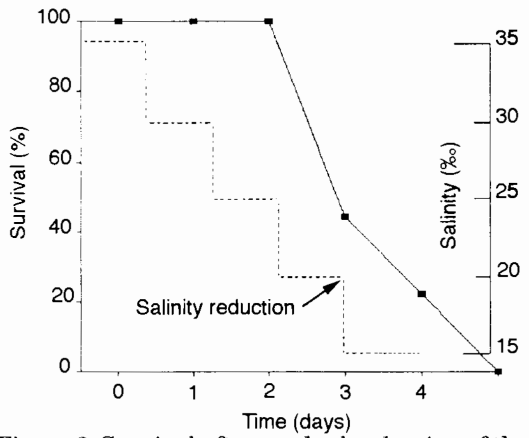

Figure 2. Survival after gradual reduction of the salinity (Exp. 2).

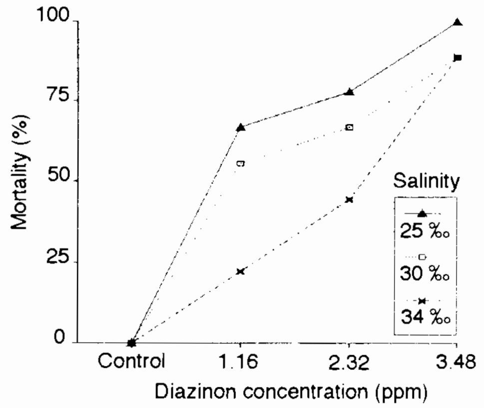

Figure 3. Effects of salinity on the toxicity of diazinon during 48 hours of exposure.

is less tolerated compared to gradual reduction. Singhagraiwan *et al.* (1992) reported that juvenile donkey's ear abalone, *H. asinina* can tolerate a salinity of 20 ‰ without acclimation and a salinity as low as 12.5 ‰ when it decreases gradually (with acclimation), at least for 14 days.

Effects of diazinon and salinity-diazinon interaction on marine invertebrates

Few relevant comparative studies have been done on the effects of diazinon on marine invertebrates (especially on molluscs). In addition, comparison with previous studies must be handled with caution because of the variability of exposure periods, test conditions and response differences within the species used.

Parathion is a related insecticide in the group of organophosphorus insecticides. Lasut (1996) found that the LC_{50} values of ethyl parathion is 1.2 and 4.5 ppm at 96 hours for larvae and adults, respectively, of the polychaete *Ophryotrocha diadema* (Dorvilleidae). He also found that the insecticide caused mortality and affected growth and reproduction of the animals. Whitten & Goodnight (1966 *in* Mulla *et al.* 1981) reported that the concentration of 5.2 ppm caused mortality for two oligochaetes, *Tubifex* sp. and *Limnodrilus* sp. Persoone *et al.* (1985) have also shown that the LC_{50} (24-96 hours) of the insecticide on crustacean and molluscs are 0.4–5.6 and 0.8-10 ppm, respectively. In our study, the LC_{50} of diazinon was 10.1, 5.2, 4.8, and 2.3 ppm at 12, 24, 36, and 48 hours respectively (Exp. 3).

Tjeerdema *et al.* (1991a, b; 1993) reported the interactive effects of pentachlorophenol and hypoxia, pentachlorophenol and temperature, and sublethal effects of the chemical on red abalone (*Haliotis rufescens* Swainson, 1822).

The interaction between diazinon and salinity on *H. varia* recorded by the diazinon-salinity test (Exp. 4), indicated no significant effect ($P > 0.05$). The toxicity of the chemical is higher at higher salinity. So, it could be speculated that the salinity at the lower point of the tolerable range (see Exp. 1 & 2) may slightly affect the condition of the test animals. The animals become more sensitive under this condition. The reduced salinity consequently caused an indirect effect on the toxicity of diazinon.

Although different experimental conditions were used by other authors, the comparison with their results are indicative of similar or related effects. Similar patterns have been found for some other marine invertebrate species: Eisler (1969) used a decapod crustacean and Monserrat *et al.* (1991) an estuarine crab *Chasmagnathus granulata* Dana, 1851 (Grapsidae) respectively. They suggested that greater salinities enhance the toxicity of organophosphorus insecticide (such as methylparathion and malathion).

ACKNOWLEDGEMENT

We are greatly indebted to the DANIDA sponsored Tropical Marine Mollusc Programme (TMMP) for the possibility to present this paper at the Seventh TMMP Workshop/Conference at Semarang, Indonesia. We are particularly grateful to our colleague Mr. I.J. Lihu who has accompanied us during the work. Our thanks are also due to Mr. B. Pratasik who gave comments on our manuscript. We also want to thank all colleagues at the Laboratory of Marine Ecology, Marine Science Department, Faculty of Fisheries University of Sam Ratulangi, Manado, Indonesia.

REFERENCES

Abel, P.D. 1991. Lethal toxicity test: theory and methodology. Pages 39-56 *in* P.D. Abel & V. Axiak (eds.). Ecotoxicology and the marine environment. - Ellis Horwood. New York.

Buhagiar, A. & P.D. Abel. 1991. Development of a computer program for analyzing toxicity test result: the "toxicologist" system. Pages 77-101 *in* P.D. Abel & V. Axiak (eds.) Ecotoxicology and the marine environment. - Ellis Horwood. New York.

Connell, D.W. & G. J. Miller. 1984. Chemistry and Ecotoxicology of pollution. Pages 162-223 *in* Pesticide (Chapter 7). - John Wiley & Sons.

Eisler, R. 1969. Acute toxicity of insecticides to a marine decapod crustacean. - Crustaceana **16**(3): 302-310.

Finney, D.J. 1971. Probit Analysis. Third edition. - Cambridge, at the university press. 333 pp.

Fowler, J. & L. Cohen. 1990. Practical Statistics for Field Biology. - John Wiley & Sons. Chichester. 227 pp.

Fuse, D.M. 1981. Notes on the biology of *Haliotis varia* and *H. asinina*. - Fisheries Research Journal of the Philippines **6**(1): 39-49.

Gallo, M.A. & N.J. Lawryk. 1991. Organic phosphorus pesticides. Pages 1049-1053 *in* W.J. Hayes, Jr. & E.R. Laws, Jr. (eds.). Handbook of pesticide toxicology. Vol. 2. Classes of pesticides. - Academic Press, Inc. Harcourt Brace Jovanovich, Publishers. San Diego.

Kaligis, G.J.F. 1994. Intertidal abalones on the coast of north Sulawesi: a survey of the distribution and abundance of *Haliotis varia* and *H. asinina*. - Phuket Marine Biological Center Special Publication **13**: 153-156.

Kobayashi, K., R.M. Rompas, T. Maekawa, N. Imada & Y. Oshima. 1990. Change in metabolic activity of tiger shrimp larvae at different stages to fenitrothion, an organophosphorus insecticide. - Nippon Suisan Gakkaishi **56**(3): 489-496.

Lasut, M.T. 1996. Toxic effects of ethyl parathion and polluted sea water on the marine polychaete *Ophryotrocha diadema* (Dorvilleidae). - M.Sc. Thesis. University of Aarhus. Denmark. 29 pp.

Monserrat, J.M., E.M. Rodriguez & R.J. Lombardo. 1991. Effects of salinity on the toxicity of parathion to the estuarine crab *Chasmagnathus granulata* (Decapoda: Grapsidae). - Bulletin of Environmental Contamination and Toxicology **46**: 569-575.

Mulla, M. S., L. S. Mian & J. A. Kawecki. 1981. Distribution, transport, and fate of the insecticides malathion and parathion in the environment. Pages 1-137 *in* F.A. Günther & J.D. Günther (eds.). Residue reviews. Residues of pesticides and other contaminants in the total environment. - Springer-Verlag. New York.

Persoone, G., P. Vanhaecke & E. Gobbers. 1985. Evaluation of the impact of parathion, methyl-parathion, fenitrothion and fenthion on the aquatic environment. - Final report EEC Contract XI/785/83, 579 pp.

Rand, G.M. & R.S. Petrocelli. 1985. Fundamentals of Aquatic Toxicology. - Hemisphere publishing corporation. New York. 666 pp.

Rodriguez, E.M. & A. Pisano. 1993. Effects of parathion and 2,4-d to egg incubation and larvae hatching in *Chasmagnathus granulata* (Decapoda: Brachyura). - Comparative Biochemistry and Physiology **104C**(1): 71-78.

Rompas, R.M., K. Kobayashi, Y. Oshima, N. Imada, K. Yamato & Y. Mitsuyasu. 1989. Relationship between toxicity and acetylcholinesterase inhibition of some thiono- and oxo-form organophosphates in tiger shrimp larvae at different stages. - Nippon Suisan Gakkaishi **55**(4): 669-673.

Sembel, D.T., F. Kaseger, J. Pongoh & D. Kandowangko. 1991. Pengkajian terhadap penggunaan pestisida oleh petani di Kab. Minahasa dan Bolaang Mongondow, Prop. Sulawesi Utara (in English: Study on the used of pesticide in the farm in Minahasa and Bolaang Mongondow, Prop. Sulawesi Utara). - Jurnal Fakultas Perikanan Unsrat **1**(4): 6-13.

Singhagraiwan, T., M. Doi & M. Sasaki. 1992. Salinity tolerance of juvenile donkey's ear abalone, *Haliotis asinina* Linné. - Thailand Marine Fisheries Research Bulletin **3**: 71-77.

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 pentachloro-phenol in the abalone (*H. rufescens*) as measured by *in vivo* 31P-NMR spectroscopy. - Journal of Biochemical 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.

ISSN 0858-3633