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Short communication:

APPLICATION OF EGGS OF THE SEA URCHIN *DIADEMA SETOSUM* IN MARINE POLLUTION BIOASSAYS

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ABSTRACT

A manual of marine pollution bioassay using sea urchin eggs is described. In Thailand, the effects of six toxicants on developmental stages of the sea urchin *Diadema setosum* differed somewhat from results with Japanese sea urchins. However, the relative toxicity at the pluteus stage was similar to previous findings, *viz.*, Cu > Zn > Ni > Cd > ABS > NH_3.

INTRODUCTION

Kobayashi (1971) proposed the use of sea urchin eggs and embryos in marine pollution bioassays. The method was later improved to enhance the sensitivity, and to simplify it by observations of the first cleavage and pluteus formation (Kobayashi, 1990).

This paper describes a manual of marine pollution bioassay using eggs of the sea urchin *Diadema setosum* (Leske) which is widely distributed over the tropical and subtropical parts of the Indo-Pacific. The species is abundant around Phuket Marine Biological Center, Phuket Island, the Andaman Sea.

MATERIALS AND METHODS

The manual for bioassay Ranking IV (Kobayashi, 1971) is presented below with some additions.

1. All instruments must be very clean.
2. Very clean plastic buckets or plastic tanks should be used to keep the test water; metal buckets should never be used.
3. Collected coastal sea water for biomonitoring purposes should be used as soon as possible, within 24 hrs at latest, or stocked in refrigerator.
4. Sea urchins must be collected at the peak of maturity, just before or after spring tide (Kobayashi, 1969); they must be used within one or two days after collection.
5. Three to five females and two to three males should be used in order to obtain enough eggs and sperms.
6. Eggs are obtained by the injection of 0.1 M acetylcholine chloride solution into the body cavity or by weak electric stimulation of the body. The eggs must be washed several times with fresh sea water, and be used as soon as possible, within 1 hour. The degree of gonadal maturity of treated urchins, and the amount of discharged eggs is noted as follows; fully ripe: +++, ripe: ++. No immature or overmature eggs should be retained in any batch.
7. The sperm should be used within 6 hrs. after removal from the tests.
8. The standard sperm density for insemination is about 1 volume of undiluted sperm to 100,000 volumes of sea water.
9. If, necessary, a preliminary check of fertilization may be performed. The fertilization membrane should be elevated within 3 minutes after insemination in at least 91% of the eggs, and the well-synchronized first cleavage should occur in at least 91% of the eggs.
10. Glass finger bowls of 5 cm in diameter and 3 cm in depth, or glass dishes of 3-4 cm in diameter, 1.5-2 cm depth should be used. The bowls should be covered with a watch glass.
11. At first, sperms are put into the test water, and eggs are added after 5 minutes.

12. The eggs should be dispersed in one layer on the bottom of a finger bowl, or dish filled with the test medium.
13. The rate and state of the first cleavage, that is, the proportions of two normal cells, undividing cells, and multi-cells caused by polyspermy are checked at adequate intervals. Two hundred eggs are fixed with 5% formaldehyde solution at a time.
14. The state of larvae (including those deposited on the bottom) and the proportions of normal and abnormal plutei (retarded, malformed, pre-pluteus embryos, embryos and larvae), are checked at adequate intervals and compared with controls. Two hundred eggs, embryos or larvae should be checked each time, and the examination repeated 3 times with different batches.
15. In grading, take the lowest figure for normal features but the highest for abnormal ones; exceptional figures should be excluded. The grade of pollution is represented by the highest grade throughout the checked indicatory features, as this will determine the survival rate (Kobayashi, 1971).
16. A ranking of sea water pollution (ranking IV) is proposed in Table 1 in accordance with Kobayashi (1990).

Table 1. Ranking of the sea water pollution by using sea urchin eggs (Ranking IV).

Inhibitory degree	Grade	First cleavage		Pluteus formation
		1 cell	Multi-cells (polyspermy)	(abnormal*)
Strong inhibition	3	50-100%	15-100%	50-100%
Moderate inhibition	2	30-49	9-14	30-49
Weak inhibition	1	10-29	3-8	5-29
No inhibition	0	0-9	0-2	0-4

* Retarded, malformed, pre-pluteus embryos and dead embryos or larvae

RESULTS AND DISCUSSION

Effects of toxicants on developmental stages of *Diadema setosum*

Heavy metals and other toxicants act on formation of the fertilization membrane, cell division, gastrulation, pluteus formation and many other developmental phenomena of sea urchin eggs. Toxicants may result in polyspermy, retardation of development, and malformation which can be used in bioassays to measure the degree of marine pollution.

Six toxicants were prepared by successive dilution of the original solution to concentrations shown in Table 2. The effects upon the stages at first cleavage and pluteus formation were in general accordance with Kobayashi (1971, 1990). The following records were made:

Cu: Higher concentrations of Cu caused eggs and embryos to develop abnormally; polyspermy induced irregular cleavage and permanent blastula. Retarded development was evident at medium concentrations in most cases; exogastrula did not appear in any cases.

Zn: Higher concentrations of Zn caused eggs and embryos to develop abnormally; eggs appeared unfertilized until cleavage began. Polyspermy, permanent blastula and exogastrula appeared at higher concentrations. Gastrula (shaped like the spaceship Apollo) appeared at medium concentrations. Retarded development was evident at weak concentrations.

Ni: Eggs and embryos developed more or less like in media with Zn, as described above.

Cd: Higher concentrations caused eggs and embryos to develop abnormally. However, few exo- and Apollo-like gastrula appeared. Developmental stages without skeleton, or with small skeletons occurred frequently.

ABS: Higher concentrations of ABS blocked development or induced cytolysis of eggs. There was no evidence of polyspermy and exogastrulae. The development was anomalous (no skeleton) or retarded even at the lower concentrations.

NH_3: The influence of NH_3 was similar to that of ABS. Higher concentrations induced cytolysis after the cleavage. Retardation of the development was evident in most cases. Exogastrula and other abnormal gastrulae did not appear.

Table 2. Effects of various chemicals on egg development in *Diadema setosum* (%). Test: 12-28 March 1991. Water temperature; 27 °C. P-B: permanent blastula, E-G: exogastrula, Apollo: the Apollo-Like gastrula.

Chemicals	Conc. (ppm)	1 st cleavage (1h) normal	1 cell	multi-cell (polyspermy)	Pluteus formation (48hrs) normal N	retarded R	malform P1	per- pluteus P2	dead embryo D	Ultimate state
Control		100	0	0	97	2	1			normal
$CuSO_4.5H_2O$	0.1	24	76							P-B
	0.05	68	28	4		6	13	81		gastrula
	0.02	74	26			86	14			retarded
	0.01	92	8		73	9	18			retarded
	0.005	95	5		98	1	1			normal
$ZnCl_2$	0.1	48	52					100		P-B
	0.05	61	37	2		12	88			P-B, E-G
	0.02	74	26			81	19			E-G, Apollo
	0.01	95	5		80	12	8			retarded
	0.005	96	4		95	3	2			normal
Control		95	5		93	5		1	1	normal
$NiCl_2.6H_2O$	10	9	91					97	3	P-B
	5	14	86				21	77	2	P-B, E-G
	2	84	11	5		8	82	4	6	E-G
	1	95	5			6	87	4	3	Apollo
	0.5	98	2		18	12	63	3	4	retarded
	0.2	96	4		92	2	3	2	1	normal
$CdCl_2.2.5\ H_2O$	5	14	86					95	5	P-B
	2	94	6			61	16	21	2	no skeleton
	1	95	5			83	11	2	4	no skeleton
	0.5	97	3		7	69	19	3	2	retarded
	0.2	98	2		91	5	1	2	1	normal
Control		95	5		98	2				normal
ABS	5	11	89							cytolysis
	2	85	15					98	2	P-B
	1	91	9			51	13	36	2	no skeleton
	0.5	95	5		73	22	2	1	2	retarded
	0.2	94	6		98	1			1	normal
NH_4Cl	10	73	27							cytolysis
	5	94	6							cytolysis
	2	92	8					96	4	P-B
	1	95	5				12	83	5	retarded
	0.5	97	3		65	21	6	5	3	retarded
	0.2	96	4		98	1			1	normal

Table 3. Estimated threshold concentrations associated with inhibition of first cleavage and pluteus formation in sea urchin eggs (Kobayashi, unpublished data).

Chemical	Concentration (unpubl.) (ppm)	Concentration (Kobayashi, 1971) (mg/l)
Hg	0.004	0.02
Cu	0.01	0.05
Zn	0.01	0.07
Ni	0.2	0.6
Cd	0.5	0.8
Al	0.5	-
Cr (VI)	1	4.2
Fe	1	-
M	2	6.6
Co	2	17
Cn	0.05	0.1
HCHO	0.1	0.4
ABS	0.5	1.6
NH_3	0.5	3
Phenol	4	15.5

Table 3 shows the effective threshold concentrations of various heavy metals and toxicants applied to sea urchin eggs or embryos in Japan. The Table lists data for inhibition of the first cleavage and pluteus formation (by Ranking III). These data indicate that the sensitivity of this bioassay method is 3 to 5 times higher than a previous method (Kobayashi, 1971). The effects of toxicants on early stages of *Diadema setosum* were somewhat different from results with Japanese sea urchins (Kobayashi, 1971, 1990). This finding may indicate that the 'nature' of the sensitivity has not yet been fully analyzed. However, the relative toxicity at the pluteal stage was similar to findings by Kobayashi (1971, 1990), *viz.*, Cu > Zn > Ni > Cd > ABS > NH_3.

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