Marine Biology 58, 163–171 (1980)

MARINE BIOLOGY

Comparative Sensitivity of Various Developmental Stages of Sea Urchins to Some Chemicals[1,2,3]

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Abstract

The sensitivity to some chemical agents was examined comparatively at sperm, fertilization, cleavage, blastula, gastrula, pluteus and metamorphosis stages of a sand dollar from Japanese waters (*Peronella japonica*) and a sea urchin from the Pacific coast of Australia (*Heliocidaris erythrogramma*). These agents included Cu sulphate, ABS and NH_3 chloride. Responses observed included departures from control rates of fertilization and developmental reduction at the attainment of first cleavage, gastrula, pluteus or metamorphosis. Developmental anomalies were noted at the fertilization, 2-cell, gastrula, pluteus and metamorphosis stages. Using minimum effective concentrations of the 3 chemicals at various developmental stages of *P. japonica*, it was found that sensitivity to chemicals varies from fertilization to metamorphosis. It seems that sperm activity is the most sensitive, and that fertilization and gastrulation are more sensitive than first cleavage, blastulation and pluteus formation. *H. erythrogramma* seems to show nearly the same responses to Cu, but is more sensitive at metamorphosis.

Introduction

There have been many investigations dealing with effects of chemicals on marine invertebrates. Most of this research has dealt with toxicity to one stage of the test animals' life history, generally embryo or adult (e.g. Waterman, 1937; Okubo and Okubo, 1962; Kobayashi, 1971 and Lönning and Hagström, 1975). The sensitivity of marine animals to environmental changes may vary during developmental processes.

In 1971 the author proposed the use of sea urchin eggs and embryos as indicatory materials in marine pollution bioassay and the method was improved to enhance the senstivity by using aged eggs (1974). Later it was found that the effects on the formation of the pluteus or metamorphosis were more pronounced than in any of the earlier stages (1977). These studies used eggs mainly of the Japanese sea urchin *Anthocidaris crassispina*. Recently, comparisons have been made of the sensitivity to various chemicals of eggs of Japanese and Canadian sea urchins (1980).

There has been very little research dealing with comparative effects of chemicals on various stages of the development of sea urchins. For example, the effect of copper-enriched seawater on fertilization, cleavage, larval skeletal development and adults of sea urchin was tested by Heslinga (1976).

This paper examines responses of sea urchin developmental stages, from fertilization to metamorphosis, to chemicals used earlier, namely Cu sulphate, alkyl benzyl sulphate (ABS) and ammonia chloride. Comparisons are made of sensitivity in the Japanese sand dollar *Peronella japonica* and a sea urchin from the Pacific coast of Australia, *Heliocidaris erythrogramma*. Both species are convenient to observe from fertilization to metamorphosis, in that *P. japonica* shows rapid development in only 3 or 4 d (Mortensen, 1921; Okazaki and Dan, 1954; Okazaki, 1975 and Kobayashi, 1977) and *H. erythrogramma* also shows a similar rapid development (Mortensen, 1921; Williams and Anderson, 1975).

Materials and Methods

1. Peronella japonica

The sand dollar, *Peronella japonica* (Mortensen), is found commonly, though not abundantly, on the

[1] Experiments conducted at the Seto Marine Biological Laboratory, Wakayama Prefecture, Japan
[2] Experiments conducted at School of Biological Sciences, the University of Sydney, Sydney, N.S.W., Australia
[3] Contributions from the Seto Marine Biological Laboratory, No. 664

0025-3162/80/0058/0163/$01.80

shallow sandy bottom in the vicinity of Seto, Japan and matures sexually in June to September. The eggs, about 300 μ in diameter, are heavily laden with yolk, opaque and pinkish. The number of eggs produced by a female at any one time are much fewer than in common sea urchins. In experiments, eggs and sperms were obtained by injection of KCl (0.5 mol solution) through the anus. The eggs were shed into seawater and deposited on the bottom. The eggs were rinsed several times with fresh sea water and experimented with as soon as possible. The sperm density at insemination was maintained at about 1 dry sperm:1000 seawater by volume.

Sperm (pretreated 3 min before fertilization), fertilization and various developmental stages were treated with respective test water samples and rates of successful fertilization, first cleavage, gastrulation, pluteus formation and metamorphosis and of some abnormalities in development were checked at 26 °C.

Series of concentrations of the chemicals to be tested were prepared by successive dilution of the original solution (chemicals --- $1{:}10^4$ – 10^3 seawater by volume = 100 – 1,000 ppm).

Firstly, the percentage of eggs with elevated fertilization membranes relative to the total eggs was read 5 min after insemination, as the elevation of the fertilization membrane was slow in this species. The first cleavage occurred in most cases about 40 min after insemination. The rate and state of the first cleavage, namely proportions of undivided cells, normal two cells and multi-cells caused by polyspermy, were then checked. About 50 eggs were checked in both cases. At the stage of the swimming embryos, proportions of permanent blastulae, normal gastrulae and exogastualae were checked in about 30 embryos about 12 h after insemination. Proportions of abnormal embryos and normal plutei were checked in about 20 embryos about 24 h after insemination. Lastly, numbers of abnormal larvae and normal juveniles were checked in about 10 larvae and juveniles about 96 h after insemination. The check was repeated 3 times in every examination on different batches in respective water samples. The normal development of the present sand dollar was described by Okazaki (1975) and Kobayashi (1977).

2. *Heliocidaris erythrogramma*

The sea urchin *Heliocidaris erythrogramma* (Val.), is commonly found on rock platforms of the open coast near Sydney, Australia and matures sexually in December to March (Williams and Anderson, 1975). Specimens used in the present investigation were collected from Balmoral and La Perouse. The egg, about 400 μ in diameter, is densely yolky, opaque and deep orange. Considerable numbers of eggs are produced by a female at a time. Eggs were obtained by injection of KCl solution into the body cavity. The eggs were shed into seawater and floated at the surface. They were rinsed several times with fresh seawater and fertilized within 1 h. Sperm were used within 1 h of removal of the test and used for insemination at the standard density of about 1 dry sperm:1000 seawater by volume.

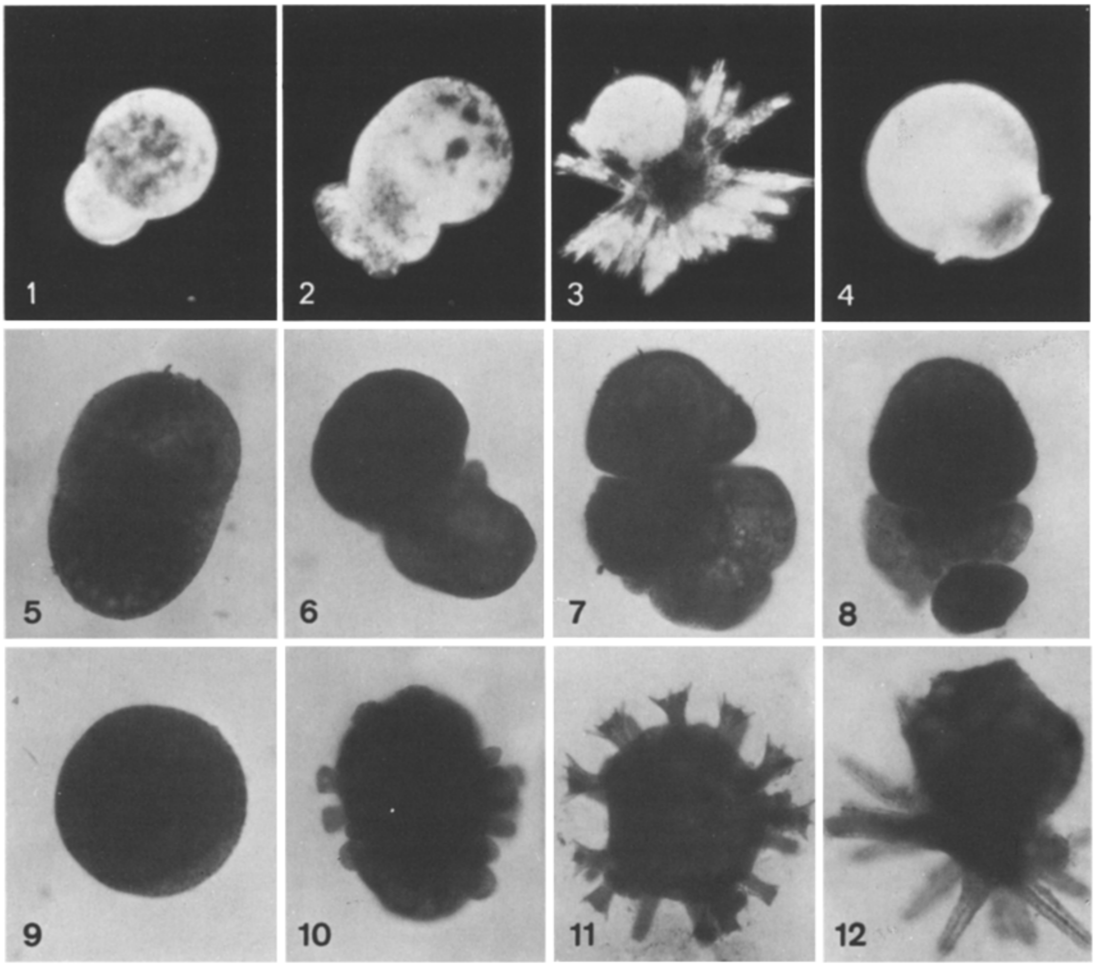
Sperm (pretreated 3 min before fertilization), the fertilization and various developmental stages were treated with Cu-containing test water samples and rates of successful fertilization, first cleavage, gastrulation and metamorphosis and of some anomalies in development were checked at 24 °C.

Firstly, the percentage of eggs with elevated fertilization membranes relative to the total eggs was read 3 min after insemination. The first cleavage occurred in most cases about 70 min after insemination. The rate and state of the first cleavage, namely proportions of undivided cells, normal two cells and multi-cells caused by polyspermy were checked. About 20 eggs were checked in both cases. At the stage of swimming embryos, the proportions of abnormal blastulae and normal swimming gastrulae with invaginations were checked in about 15 embryos about 20 h after insemination. The gastrula was elongated and swam freely, then became a postgastrula. The larvae without arms were fully developed in 2 d and showed an ovoid shape or a doubly annular-constricted shape between the preoral lobe and the echinoid rudiment and between the rudiment and the posterior lobe. Larvae having these constrictions often shed posterior yolk lobe or the anterior (Figs. 1,5). The echinoid rudiment of the free-swimming lecithotrophic larva entered metamorphosis in 2.5 d (Figs. 2,6). The metamorphosing larva settled and became a juvenile urchin in 4 to 5 d (Figs. 3, 10 to 12). Abnormal larva which failed to metamorphize was observed in Cu-solution (Figs. 4, 7 to 9). Numbers of abnormal larvae and normal juvenile were checked in about 10 larvae and juveniles.

The check was repeated 3 times in every examination on different batches in Cu-containing water samples. Normal developmental features in the present sea urchin were described in detail by Williams and Anderson (1975). The density of embryos decreased with the development. In case this might be partly due to the contamination of the test water by dead embryos, these dead embryos were removed twice daily by pipetting.

Results

The response to Cu, ABS and ammonia from fertilization to metamorphosis of eggs or embryos of *Peronella japonica* and *Heliocidaris erythrogramma* are summarized in Tables 1 to 4. In general, the responses noted compare with those obtained for other species (Kobayashi, unpublished data), although there are suggested differences in species sensitivity. In the sand dollar, the normal development in the present examination was defined by the pluteus formation of 80% or more of gastrulae and the successful metamorphosis of 65% or more of plutei. In the sea urchin, the rates of various stages of normal development were somewhat lower than the sand dollar.



Figs. 1 to 12. Normal and abnormal developmental stages from larva to metamorphosis in the experiments with *Heliocidaris erythrogramma*. 1–4: × 50, 5–12: × 60. Experiments were performed at a water temperature of 24 °C. The normal stages were observed in normal sea water. 1–4: Living materials, 5–12: Fixed materials. 1. Normal early larva without posterior lobe:constricted shape, 2 d after insemination. 2. Early metamorphosis, 2.5 d after. 3. Juvenile urchin, 8 d after. 4. Abnormal larva failed metamorphosis in Cu-seawater, 5 d after. 5. Normal early larva, ovoid shape, 2 d after. 6. Early metamorphosis, 2.5 d after. 7. Abnormal metamorphosis in Cu-seawater, 4 d after. 8. Abnormal metamorphosis in Cu-seawater, disintegrating, 4 d after. 9. Abnormal larva failed development in Cu-seawater, 5 d after. 10. Normal late metamorphosis, 3.5 d after. 11. Normal juvenile urchin, 5 d after. 12. Normal Juvenile urchin, 5 d after

Cu

In the higher concentrations of Cu, the eggs or embryos of both species remained intact or developed somewhat abnormal states. The responses appeared stronger in early stages of development than in later stages. Polyspermy did not occur at all concentrations in either species. Four or 5 days after treatment, responses to Cu had extended to lower concentrations; the responses differed among the various developmental stages treated. Retarded development was evident in almost all cases, but exogastrula did not appear in all cases.

ABS

Higher concentrations of ABS reduced development. There was no evidence of polyspermy. At 4 days, development was anomalous or retarded in lower concentrations. The responses differed in the various developmental stages treated, and exogastrula did not appear in all cases.

Ammonia

Quantitatively, the influence of ammonia was similar to that of ABS. Some reduction in fertilization membrane formation occurred at the highest concentrations treated before and at fertilization. There was no evidence of polyspermy or exogastrula formation.

In summary, at metamorphosis the relative toxicity of the 3 compounds tested was Cu>ABS>NH_3.

Table 1. *Peronella japonica*. Effects of Cu on various developmental processes. Tests: July; water temperature: 26 °C

Processes (after fertilization)	Conc.	Time after insemination									
		5min	40min		12 h		1 d		4 d		Ultimate state
		Fert.memb. formation	1 cell state	2 cell stage	Arrest. blast.	Normal gast.	Abnormal embryo	Normal pluteus	Abnormal larva	Normal juven.	
	ppm	%	%	%	%	%	%	%	%	%	
Control		95 (90±5)	15	85 (90±5)	5	95 (90±5)	10	90 (85±5)	25	75 (70±5)	normal
	0.2	15	90	10							8–16 cells
	0.1	20	80	20							32 cells
Sperm	0.05	30	75	25	100	0					blastula
– 3 min	0.02	55	55	45	100	0					blastula
	0.01	85	20	80	35	65	40	60	55	45	retardation
	0.005	95	10	90	10	90	15	85	40	60	normal?
	0.2	25	85	15							16–32 cells
	0.1	35	80	20							32 cells
Fertiliz.	0.05	55	65	35	80	20					blastula
0 min	0.02	70	40	60	35	65	100	0			retardation
	0.01	85	15	85	15	85	20	80	35	65	normal
	0.2		10	80							16–32 cells
Cleavage	0.1		15	85							32 cells
30 min	0.05		15	85	100	0					blastula
	0.02		10	90	15	85	20	80	35	65	normal
	0.2				100	0					blastula
Swimming	0.1				70	30	100	0			blastula
Blastula	0.05				40	60	100	0			retardation
8 h	0.02				10	90	20	80	35	65	normal
	0.2						100	0			gastrula
	0.1						100	0			gastrula
Gastrula	0.05						90	10			retardation
12 h	0.02						35	65	100	0	retardation
	0.01						20	80	40	60	normal?
	0.005						10	90	30	70	normal
	0.2								100	0	pluteus
Pluteus	0.1								100	0	pluteus
24 h	0.05								40	60	retardation
	0.02								30	70	normal

Comparative Sensitivity

Cu

Peronella japonica. In the sperm (pretreated before fertilization) and the fertilization tests, concentrations which reduced the fertilization membrane formation of the control level were lower in the sperm test that in the fertilization one. In the reduction of the two cell stage, the fertilization test appeared less sensitive than the sperm test, as in the case of reduced fertilization membrane formation. In the reduction of gastrulation, the sensitivity of the preceding processes appears in the order, sperm>fertilization>cleavage>blastula. In the reduction of pluteus formation, the sensitivity of the preceding processes appears in the order, sperm>fertilization>gastrulation>cleavage>blastula. Gastrulation is thus more sensitive than the other 2 processes. In the reduction of the juvenile formation, the sensitivity appears in the order, sperm>gastrulation>fertilization>cleavage, blastula>pluteus. On the other hand, according to the development in each test, responses were gradually increased at each stage.

Heliocidaris erythrogramma. In the reduction of fertilization membrane formation, the sperm test is more

Table 2. *Peronella japonica*. Effects of ABS on various developmental processes. Tests: July; water temperature 26 °C

Processes (after fertilization)	Conc.	Time after insemination									
		5 min	40 min		12 h		1 d		4 d		Ultimate state
		Fert.memb. formation	1 cell state	2 cell stage	Arrest. blast.	Normal gast.	Abnormal embryo	Normal pluteus	Abnormal larva	Normal juven.	
	ppm	%	%	%	%	%	%	%	%	%	
Control		95 (90±5)	5	95 (90±5)	0	100 (90±5)	15	85 (85±5)	30	70 (70±5)	normal
	5	10	95	5							32 cells
	2	80	25	75	25	75	100	0			gastrula
Sperm	1	90	10	90	15	85	85	15	100	0	retardation
– 3 min	0.5	95	5	95	20	80	65	35	100	0	retardation
	0.2	90	10	90	10	90	45	55	45	55	normal?
	0.1	95	5	95	5	95	15	85	35	65	normal
	5	25	85	15	90	10					blastula
	2	70	35	65	20	80	95	5			gastrula
Fertiliz.	1	95	10	90	15	85	80	20	100	0	retardation
0 min	0.5	95	5	95	10	90	20	80	85	15	retardation
	0.2	90	10	90	5	95	20	80	35	65	normal
	5		5	95	20	80	90	10			gastrula
Cleavage	2		10	90	15	85	75	25			gastrula
30 min	1		5	95	5	95	20	80	30	70	normal
	5				30	70	100	0			gastrula
Swimming	2				20	80	65	35			retardation
Blastula	1				5	95	20	80	35	65	normal
8 hrs											
	5						100	0			retardation
Gastrula	2						100	0			retardation
12 h	1						45	55	40	60	normal?
	0.5						15	85	35	65	normal
	5								100	0	retardation
Pluteus	2								100	0	retardation
24 h	1								70	30	retardation
	0.5								30	70	normal

sensitive than the fertilization test. The same relationship occurs in the arrest of the 2 cell stage. In the reduction of gastrula, sensitivity seems to be in the order, sperm>fertilization>cleavage>blastula. In the reduction of the juvenile, sensitivity appears to be in the order, sperm>gastrula>metamorphosis>fertilization>blastula>cleavage. Sensitivities of the gastrula and the metamorphosis stage are considerable. On the other hand, according to the development in each test, responses were gradually increased at each stage.

ABS

Peronella japonica. In the reduction of fertilization membrane formation, the sperm test is more sensitive than the fertilization test. In the reduction of the 2 cell stage, the same phenomenon occurs, In the reduction of the gastrula, sensitivity appears in the order, sperm>fertilization>blastula>cleavage. In the reduction of the pluteus, sensitivity seems in the order, sperm>fertilization>gastrula>blastula>cleavage. In the reduction of the juvenile, sensitivity of the tests appears in the order, sperm>fertilization>pluteus>gastrula>blastula>cleavage. On the other hand, according to the development in each tests, responses were gradually increased at each stage.

NH_3

Peronella japonica. In the reduction of fertilization membrane formation, the sperm test is more sensitive than the fertilization test. In the reduction of the 2

Table 3. *Peronella japonica*. Effects of NH_3 on various developmental processes. Tests: July; water temperature: 26 °C

Processes (after fertilization)	Conc.	Time after insemination									
		5 min	40 min		12 h		1 d		4 d		Ultimate state
		Fert.memb. formation	1 cell state	2 cell stage	Arrest. blast.	Normal gast.	Abnormal embryo	Normal pluteus	Abnormal larva	Normal juven.	
	ppm	%	%	%	%	%	%	%	%	%	
Control		95	5	95	0	100	5	95	25	75	normal
		(90±5)		(90±5)		(90±5)		(85±5)		(70±5)	
	10	35	70	30	100	0					blastula
	5	95	5	95	50	50	100	0			gastrula
Sperm	2	90	10	90	35	65	90	10			gastrula
– 3 min	1	95	5	95	10	90	25	75	100	0	retardation
	0.5	95	5	95	5	95	20	80	75	25	retardation
	0.2	95	5	95	5	95	5	95	35	65	normal
	10	45	60	40	100	0					blastula
	5	90	5	95	40	60	100	0			gastrula
Fertiliz.	2	95	10	90	30	70	55	45	100	0	retardation
0 min	1	95	5	95	5	95	20	80	80	20	retardation
	0.5	95	5	95	5	95	15	85	30	70	normal
	10		15	85	100	0					blastula
Cleavage	5		15	85	45	55	100	0			gastrula
30 min	2		5	95	15	85	45	55	75	25	retardation
	1		5	95	10	90	20	80	35	65	normal
	10				15	85	95	5			gastrula
Swimming	5				10	90	80	20			gastrula
Blastula	2				10	90	55	45	80	20	retardation
8 h	1				5	95	20	80	35	65	normal
	10						100	0			gastrula
	5						80	20			gastrula
Gastrula	2						5	95	80	20	retardation
12 h	1						10	90	60	40	retardation
	0.5						5	95	30	70	normal
	10								100	0	retardation
Pluteus	5								75	25	retardation
24 h	2								30	70	normal

cell stage, sensitivity appears in the order, sperm>fertilization. In the reduction of the gastrula, sensitivity seems in the order, sperm>fertilization>cleavage. In the reduction of the pluteus, sensitivity appears in the order, sperm>fertilization>cleavage, blastula>gastrula. In the reduction of the juvenile, sensitivity of the tests is, sperm>fertilization>gastrulation>blastula>cleavage>pluteus. On the other hand, according to the development in each test, responses were gradually increased at each stage.

Discussion and Conclusions

Comparative Sensitivity of Developmental Processes

There has been very little research dealing with the comparative effects of chemicals on various stages of the development of sea urchins. The author found in these experiments that, in reducing the fertilization and further development, the sperm test is more sensitive than the other processes. K. Okubo (1973, unpublished

Table 4. *Heliocidaris erythrogramma*. Effects of Cu on various developmental processes. Tests:March; water temperature: 24 °C

Processes (after fertilization)	Conc.	Time after insemination							
		3min	70 min		20 h		5 d		Ultimate state
		Fert.memb. formation	1 cell state	2 cell stage	Arrest. blast.	Normal gast.	Abnormal larva	Normal juven.	
	ppm	%	%	%	%	%	%	%	
Control		80 (80±5)	20	80 (80±5)	15	85 (80±5)	30	70 (70±5)	normal
	0.2	0	100	0					unfertilized
	0.1	5	100	0					unfertilized
	0.05	10	100	0					1 cell state
Sperm	0.02	25	80	20	100	0			blastula
– 2 min	0.01	60	75	25	90	10			blastula
	0.005	70	50	50	35	65	80	20	retardation
	0.002	70	20	80	30	70	45	55	normal?
	0.001	75	25	75	15	85	35	65	normal
	0.2	10	100	0					unfertilized
Fertiliz.	0.1	5	100	0					unfertilized
0 min	0.05	25	95	5					1 cell state
	0.02	50	60	40	40	60	65	35	retardation
	0.01	80	25	75	20	80	35	65	normal
	0.2		25	75	100	0			blastula
Cleavage	0.1		20	80	100	0			blastula
30 min	0.05		20	80	20	80	100	0	retardation
	0.02		25	75	20	80	30	70	normal
Control		85 (80±5)	20	80 (80±5)	15	85 (80±5)	25	75 (70±5)	normal
	0.2				100	0			blastula
Blastula	0.1				40	60	80	20	retardation
10 h	0.05				25	75	50	50	retardation
	0.02				15	85	40	60	normal?
	0.01				20	80	25	75	normal
	0.2				20	80	100	0	gastrula
Swimming	0.1				15	85	100	0	gastrula
Gastrula	0.05				15	85	100	0	gastrula
15 h	0.02				20	80	65	35	retardation
	0.01				15	85	40	60	normal?
	0.005				15	85	30	70	normal
	0.2						100	0	larva
Metamorphosis	0.1						100	0	larva
2.5 d	0.05						100	0	larva
	0.02						60	40	retardation
	0.01						40	60	normal?
	0.005						25	75	normal

data) observed that the sperm test is more sensitive than the fertilization test in the action of oil dispersant on sea urchin eggs (*Anthocidaris crassispina* and *Hemicentrotus pulcherrimus*). Renzoni (1974) reported that, in measuring the influence of some toxicants on larvae of *Paracentrotus lividus* etc., the most sensitive to the various hydrocarbon/dispersant mixtures was the male gamete. Straughan (1976) showed that tolerance to high levels of dissolved petroleum in seawater is greater between the 4 cell and blastula stages than prior to the 4 cell stage and after the blastula to the pluteus stage of the sea urchin *Strongylocentrotus purpuratus*. Heslinga (1976) reported that the effects of Cu-enriched seawater on fertilization success, early cleavage, larval

Table 5. Estimated threshold concentrations of chemicals associated with reduced development in sea urchin eggs (Cu, Zn, ABS and NH_3)

Developmental processes / Chemicals	ppm	Sperm (before fertil.)	Fertilization	Cleavage (first)	Blastula	Gastrula	Further stages
		–2-3 min	0 min	30 min			
Peronella japonica	26 °C				5 h	12 h	Pluteus 24 h
$CuSO_4 \cdot 5H_2O$		0.005	0.01	0.02	0.02	0.005	0.02
$ZnCl_2$		0.005	0.01	0.02	0.05	0.005	0.05
ABS		0.1	0.3	1.0	1.0	0.5	0.5
NH_4Cl		0.2	0.5	1.0	1.0	0.5	2.0
Heliocidaris erythrogramma	24°C in Australia				10 h	15 h	Metamorphosis 2.5 d
$CuSO_4 \cdot 5H_2O$		0.001	0.01	0.02	0.01	0.005	0.005
Hemicentrotus pulcherrimus	17 °C (warmed)				12 h	24 h	1 yr adult 72 h
$CuSO_4 \cdot 5H_2O$		0.01	0.05	0.1	0.1	0.05	1
$ZnCl_2$		0.02	0.05	0.1	0.2	0.05	5
ABS		0.2	1.0	2.0	2.0	1.0	100
NH_4Cl		0.5	1.0	2.0	1.0	0.5	100
Anthocidaris crassispina	26 °C				4 h	12 h	
$CuSO_4 \cdot 5H_2O$		0.02	0.05	0.1	0.1	0.05	
$ZnCl_2$		0.02	0.05	0.1	0.1	0.05	
ABS		0.5	1.0	5.0	2.0	1.0	
NH_4Cl		1.0	2.0	5.0	5.0	2.0	
Pseudocentrotus depressus	17 °C				10 h	19 h	
$CuSO_4 \cdot 5H_2O$		0.015	0.03	0.06	0.06	0.03	
ABS		0.5	1.0	2.0	2.0	1.0	

skeletal development, and survivorship of adults of the sea urchin *Echinometra mathaei* were tested. Fifty percent reduction of fertilization success and cleavage success to the 8-cell stage occurred in 0.18 and 0.42 ppm added Cu, respectively. Adults had respective 48- and 96 h TL50 values of 0.54 and 0.30 ppm. Larval skeletal development was suppressed in 0.02 ppm. He concluded that the latter process appears to be very sensitive and may be the most suitable for assessing the effects of stress on this species.

Comparison with the author's experiments on *Hemicentrotus pulcherrimus* eggs (see Table 5) shows that the fertilization and the cleavage tests are more sensitive than Heslinga's results with similar tendencies. However, the later and the adult tests show low sensitivities.

Comparative Sensitivity of Sea Urchins to Chemicals

To examine the comparative effects of chemicals on various stages of the development of two urchins, estimated threshold concentrations for the reduction of development were compared. With respect to the effect of Cu on developmental processes, responses with similar tendencies were observed between *Peronella japonica* and *Heliocidaris erythrogramma*. Comparing the effects of Cu, ABS or NH_3 with each other on the developmental processes of *P. japonica*, the responses were similar in early stages but differed in later stages, especially in the gastrulation process.

Estimated threshold concentrations to four chemicals associated with reduced development in five species of sea urchins are shown in Table 5. Unpublished data of the author on *Hemicentrotus pulcherrimus, Anthocidaris crassispina* and *Pseudocentrotus depressus* are included. On young adults (1 yr old) of *H. pulcherrimus* ranges from 1.5 to 2.0 mm test diameter were observed to determine the effects of exposure to chemicals on spine movement and adhesion to glass walls.

The sensitivity of developmental processes to chemicals on the 5 species appears similar, but possibly demonstrates an order of sensitivity, sperm>gastrula> fertilization>blastula>cleavage. Furthermore, the metamorphosis test may have the same sensitivity as the gastrula test, while the pluteus test seems to be of the same sensitivity as the blastula or the cleavage test. The young adults are the most insensitive. The sensitivity of the development of the sea urchins to chemicals appears similar, but may be in the order *Heliocidaris erythrogramma*>*Peronella japonica*>*Pseudocentrotus depressus*>*Hemicentrotus pulcherrimus*>*Anthocidaris crassispina*. For the 4 chemicals compared, toxicity appears to be in the order Cu>Zn>ABS>NH_3. Okubo (1973, unpublished data) observed that the sensitivity of the fertilization of sea urchin eggs to oil dispersants was in the order *A. crassispina*>*H. pulcherrimus* >*P. depressus*.

The estimated threshold concentration of Cu for *Heliocidaris erythrogramma* is 0.001 ppm. However, the concentration of Cu in the open coast of Sydney is about 0.003 ppm, so that the actual concentration of Cu in the test water used was about 0.004 ppm. It appears that extremely low concentrations have an affect on *H. erythrogramma*. The higher sensitivity may also be caused by the experimental conditions of the sea urchins used.

The results of the present experiments with *Peronella japonica* and *Heliocidaris erythrogramma* show clearly that the effects of various polluted seawater are more pronounced in these species than in other species that have been tested.

Acknowledgements. The author acknowledges with great thanks the assistance provided by Professor D. T. Anderson and Dr. A. J. Underwood, School of Biological Sciences, The University of Sydney, N.S.W. Australia, for experimental facilities, test individuals and advice; and Professor E. Harada and the staff of the Seto Marine Biological Laboratory, Japan, for advice and use of experimental facilities.

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Date of final manuscript acceptance: June 10, 1980.
Communicated by M. Anraku, Nagasaki