Abnormality of Development in *Strongylocentrotus intermedius* (A. Agassiz) Larvae from Polluted Habitat in Amursky Bay, Peter the Great Bay.

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Abstract Amursky Bay, Peter the Great Bay, Sea of Japan is very much prone to anthropogenic pollution by heavy metals, oils, phenols, pesticides, etc. To clarify the effect of pollution, tests of the embryonic and larval development of sea urchin *Strongylocentrotus intermedius* are conducted using material collected from polluted habitats in Amursky Bay. A population from an unpolluted site in Vityaz Bay is used as a control. Various abnormal patterns of development including failure to metamorphose are detected. Thus sea urchin population inhabiting Amursky Bay can not reproduce normal and viable offspring, which will be ascribed to an anthropogenic influence from Vladivostok city.

Key words: sea urchin, *Strongylocentrotus intermedius*, polluted habitat, Amursky Bay, abnormalities of development, metamorphosis.

Introduction

Sea urchin gametes and embryos provide a convenient test-object for the biological effect of chemicals and pollutants and ecotoxicological testing in the marine environment (Kobayashi, 1971, 1984, 1991; Dinnel & Stober, 1987; Dinnel et al., 1988). Usually for such experiments adults are taken from unpolluted habitat. For studying the toxicity of different chemicals or medications small quantities of them are added to the clean sea water where fertilization and development takes place; for ecotoxicological experiments fertilization and subsequent development are observed in the water taken from areas to be examined. Toxicity is estimated by the percentage of gamete fertilization and percentage of abnormal embryos and larvae in the samples compared with the control.

It was noticed that sea urchin adults inhabiting an area polluted by heavy metals in the Red Sea have a deformed shell with a reduced number of interambulacral plates (Dafni, 1980). Amursky Bay, Peter the Great Bay, Sea of Japan is very much prone to anthropogenic pollution by heavy metals, oils, phenols, pesticides, etc., but no special studies on possible abnormalities of adult sea urchin were conducted. The purpose of the present study is to elucidate the degree of pollution by an ecotoxicological study using a sea urchin development as a marker. The development of embryos and larvae of *Strongylocentrotus intermedius* inhabiting Amursky Bay was investigated from fertilization to metamorphosis on the Experimental Marine Station "Vityaz" in 1984–1985.

Material and Methods

Area studied: Amursky Bay is one of the most polluted area in Peter the Great Bay (Vladivostok Center of Control of Pollution Environment, 1985–1988). Over 150,000 m^3 of industrial and municipal sewage water from Vladivostok city is discharged into the Bay daily without proper purification (Vladivostok Center of Control of Pollution Environment,

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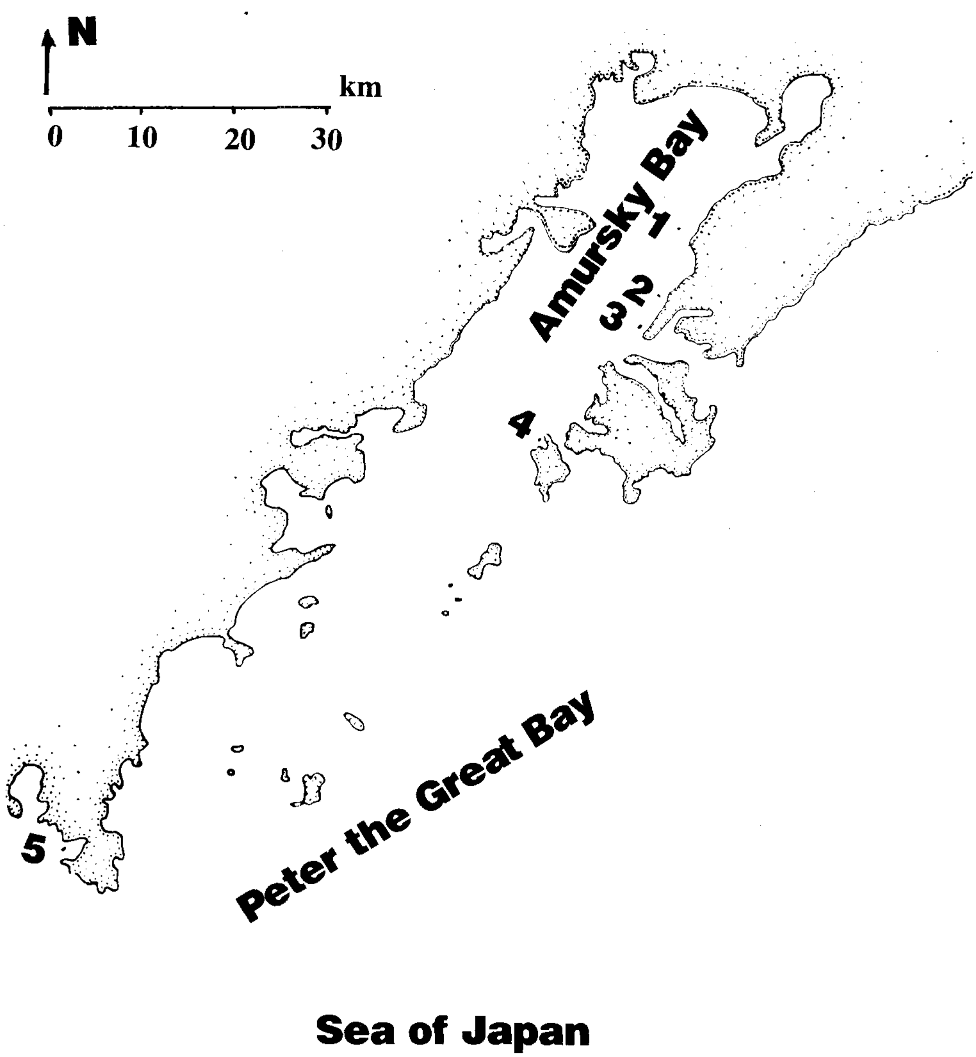

Fig. 1 Sites in Peter the Great Bay (Sea of Japan). Amursky Bay: 1 - Screbtsov Island, 2 - Sportivnaya Harbour, 3 - Egersheld Cape; 4 - Popov Island (Alekseeva Bay); 5 - Vityaz Bay.

1986). The heavy metals, oil products, phenols, synthetic surface active substances, pesticides, etc. have been detected there. During 1984–1985 the contents of oil hydrocarbons in the sea water column of Amursky Bay varied from 0 to 0.44 mg/l; those of phenols 0–0.20 mg/l; those of synthetic surfactants 0–0.25 mg/l and those of pesticides (DDT) 0–21.30 ng/l. Maximum concentrations of heavy metal ions (Cu, Co, Ni) exceeded Russian permissible values by 1.3–1.8 times (Vladivostok Center of Control of Pollution Environment, 1985, 1986). The content of total nitrogen in the water column varied from 19.40 to 994.00 mg/l, total phosphorus from 0 to 59.00 mg/l at the surface and 0.50 to 115.00 mg/l near the bottom. Sea water pollution also leads to an accumulation of toxicants in the bottom sediment; at some sites in Amursky Bay levels of heavy metals (Zn, Pb, Cd, Cr, Cu) in the bottom sediment are 1.5–2.5 times higher than background levels (Shulkin & Borisovets, 1989). According to the content of toxicant substances in water column and bottom, sampling sites in Amursky Bay are classified as belonging to pollution category 1–2, while the control site in Vityaz Bay situated in a more clear, open area in Peter the Great Bay as category 3 (Vladivostok Center of Control of Pollution Environment, 1985–1988).

Material: In August 1984, random samples of *S. intermedius* taken from 4 polluted sites in Amursky Bay and from one control site 5 in Vityaz Bay (Fig. 1), were kept at 2–3°C during several hours shipping to the Experimental Marine Station "Vityaz". After delivery the samples from different sites were kept in separate tanks with running water during 24 hours at 18–20°C. Each sample consisting of 50 adult specimens were 45–65 mm in diameter.

Methods: The best three to five parental pairs were selected from each sample. The reason of this selection is to obtain quantity of gametes from the gonads, stimulating the aboral surface of body with 8–12 V electric current (Naidenko, 1984). At first gonads were examined under light microscopy followed by histological analysis as was described by Vashchenko et al. (1992). Levels of copper, zinc, iron and manganese in the same samples were determined and already reported (Busev, 1985). The development from fertilized eggs to pluteus stage was observed in 2 l volume glass vessels filled with clean filtered water at 18–20°C according to the methods by Buznikov & Podmarev (1990). Each experiment was run in two replicates. Then 3-days-old larvae of parental pairs from each site were pooled together and then raised in a mixed culture in order to characterize the general state of larvae from the given site. Subsequent development until metamorphosis was carried out using the technique published earlier (Naidenko, 1983, 1984). The following stages were selected as indicatory ones: fertilized egg, 2 cell stage, blastula, 4,6,8-armed pluteus. For microscopic examination at least 100 eggs, embryos or larvae were taken. Extreme and average values of percentage of larvae on different developmental stages were calculated. Development and the condition of the larval cultures were monitored over 45 days. Embryos and larvae were classified according to Kawamura (1970), Kryuchkova (1976) and Buznikov & Podmarev (1990). Larval development from polluted sites is compared with normal development from the control site. Same study was repeated a year later and the results are combined.

Results

The condition of gonads at different sites

Microscopic analysis of gonads has shown that their condition was varying according to sites. Sea urchins from the control site 5 had large, bright, yellow gonads which were mostly immature; the percentage of fertilized eggs of separate parental pairs varied from 79.0 to 93.0 % (Tables 1, 2; Fig. 2 A, B). In females from sites 1 and 3 the gonads were small, shrunkn, dirty yellow or black colored; they contained small oocytes of varying size and a lot of damaged cells, conglomerates of eggs and their fragments (Fig. 3). Extreme quantity of eggs released by electrostimulation of females from different sites are shown in Table 1. Best female from site 1 did not release more than 20,000 eggs but fertilizaton did not occur despite spermatozoa released by males from the same site still preserved motility. Of 11 females from site 3 only three had some quantity of mature eggs in the gonads and could be selected for experiments. The proportion of fertilized eggs released from these females varied from 1.0 to 35.0 % (Table 2; Fig. 4). The gonads of specimens from sites 2 and 4 were large and yellow and the largest number of fertilized eggs per female was from 41.0 to 96.0 % for site 2 and from 10.0 to 59.0 % for site 4 (Table 2).

Development of embryos and larvae from different sites

Embryogenesis occurred normally and synchronously only in the control culture (Fig. 2). In cultures from polluted sites fertilization and early development were retarded. In the control culture embryos began to spin inside membranes within 7 h after fertilization, but in the cultures from polluted sites, the spinning did not take place by this time and

Table 1. Condition of gonad and fecundity of *Strongylocentrotus intermedius* from different sites of Peter the Great Bay, Sea of Japan (August, 1984).

Site	Number of females examined	Quantity of eggs from one female per one shedding, min - max	Condition of female gonads
Amursky Bay			
1	17	few - 20,000	small oocytes, damaged cells, conglomerates of eggs and their fragments
2	17	17,000 - 890,000	premature oocytes, eggs
3	11	few - 250,000	the same as in site 1
Alekseeva Bay			
4	18	150,000 - 160,000	the same as in site 2
Vityaz Bay (Control)			
5	18	150,000 - 160,000	premature oocytes, eggs

Table 2. Variability in development of *Strongylocentrotus intermedius* from different sites of Peter the Great Bay (five parental pairs from each site) (August-September, 1984).

Site	Stage, %, min-max, (average); time after fertilization				
	Membrane formation 5 min	First cleavage 70 min	Blastula 13 h	Pluteus 3 days	Final stage
Amursky Bay					
1	0	0	0	0	0
2	41.0–96.0 (69.8)	1.0–65.0 (26.8)	36.0–95.0 (70.3)	30.0–90.0 (68.0)	8-armed abnormal plutei, 48 days
3	1.0–35.0 (14.9)	0–50.0 (17.6)	0–59.0 (20.7)	0–50.0 (20.0)	4-armed abnormal plutei, 27 days
Alekseeva Bay					
4	10.0–59.0 (34.8)	29.0–53.0 (39.7)	60.0–85.0 (76.0)	60.0–80.0 (76.0)	6, 8-armed abnormal plutei, 45 days
Vityaz Bay (Control)					
5	79.0–93.0 (84.8)	54.0–74.0 (64.0)	59.0–87.0 (75.2)	80.0–95.0 (90.0)	8-armed normal plutei, 35 days; metamorphosis, 38–40 days

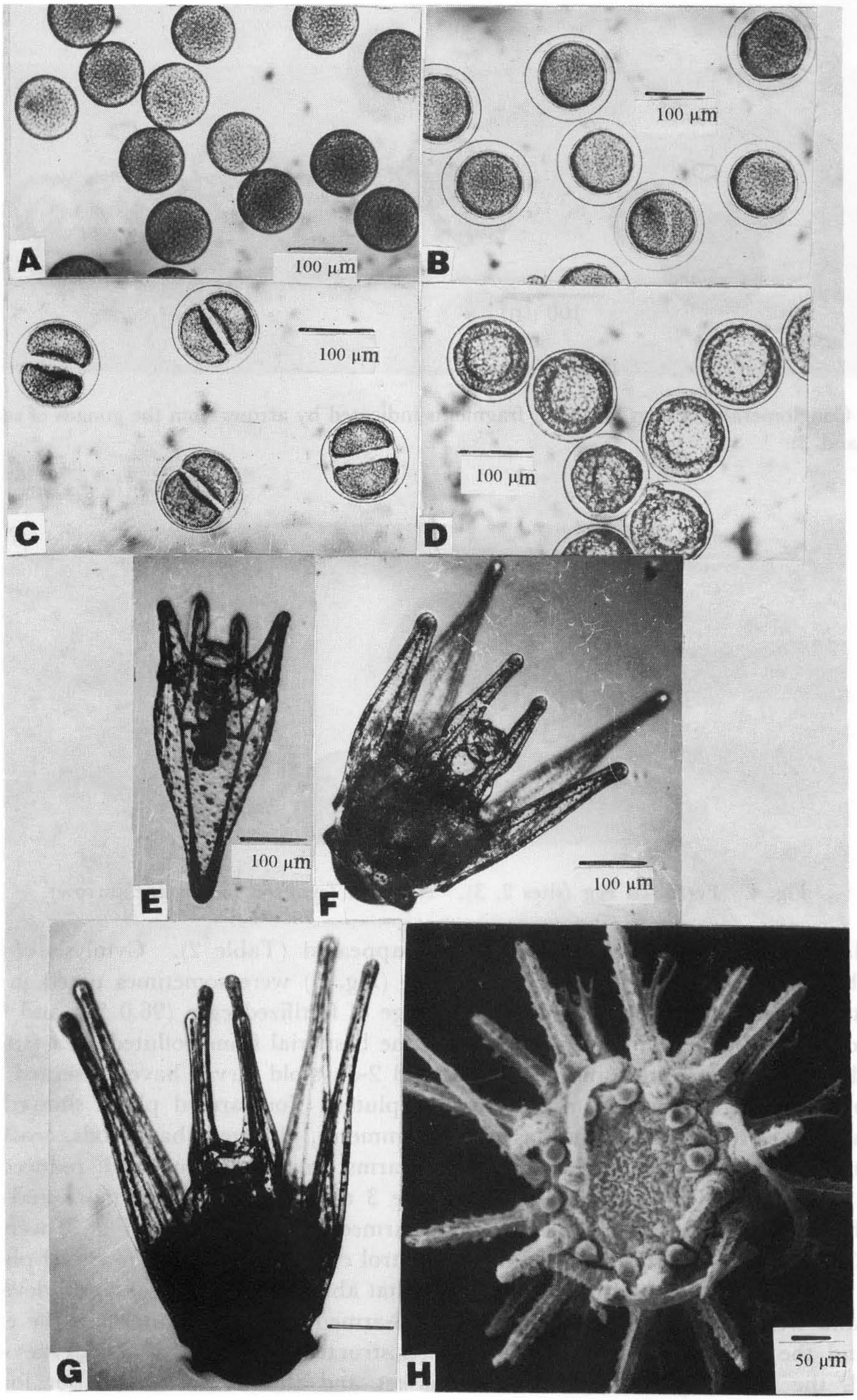

Fig. 2. Normal development of *Strongylocentrotus intermedius* (Vityaz Bay, control site 5). A - unfertilized eggs. B - fertilized eggs C - 2-cell stage. D - blastula stage. E - 4-armed pluteus. F - 6-armed pluteus. G - 8-armed pluteus. H - juvenile sea urchin after metamorphosis. Scale bar G = 115 μm.

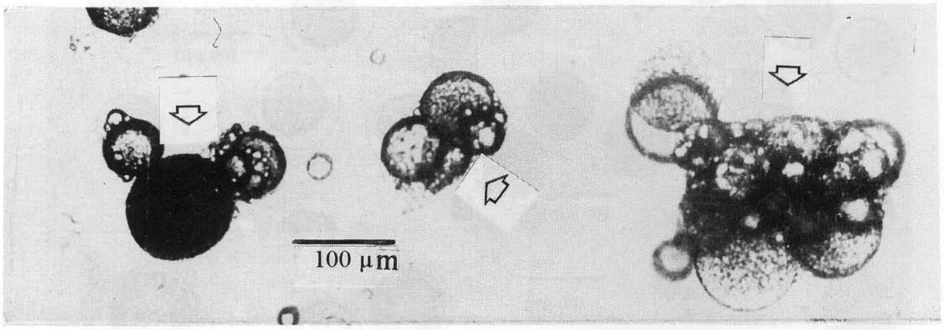

Fig. 3. Conglomerates of eggs and their fragments indicated by arrows from the gonads of sea urchins (sites 1 and 3).

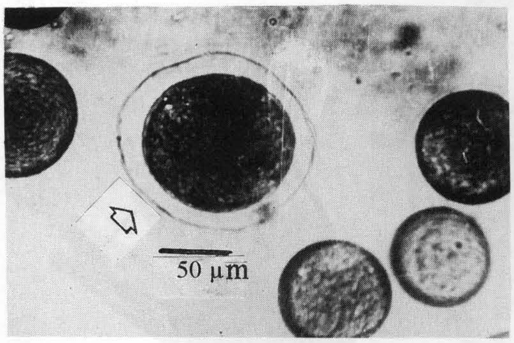

Fig. 4. Fertilized egg (sites 2, 3). Note the fertilized membrane (arrow).

variability, and abnormalities of development appeared (Table 2). Cytolysis of embryos at the blastula stage (Fig. 5) and exogastrulae (Fig. 6) were sometimes noted in cultures from sites 2 and 3. Although a high percentage of fertilized eggs (96.0 %) and blastulae formation (95.0 %) were initially observed in the best trial from polluted area (site 2), the larval development was also unsynchronical and 2-days-old larvae have presented different developmental stages from prisms to 4-armed plutei. Four-armed plutei showed various abnormalities of development such as body asymmetry, divergent basal rods, crossed basal rods, ugly swollen bodies, irreversibly bent arms, reduced arms and reduced bodies (Fig.7). Four-armed abnormal plutei from site 3 did not develop anymore and died on the 27th day. In cultures from sites 2 and 4 6-armed abnormal plutei (Fig. 8) were formed within 30 day after fertilization while in the control culture the normal 6-armed plutei were formed within 15–18 days. It should be noted that abnormal patterns of larval development varied and were not site dependent. Six and 8-armed abnormal plutei had the elongated bases and the irregular arms; the club-shaped structures at the base of larvae were not resolved, the lower epaulettes, the basal basket and the rudiment did not form (Fig. 8). Subsequent development of larvae from polluted habitat was arrested because of various structural deformities of the body. In the culture from site 4, only a small number of incompletely formed plutei 45 day old had spicules of the definitive skeleton, but none of them metamorphosed further. Therefore larvae from polluted sites were not able to

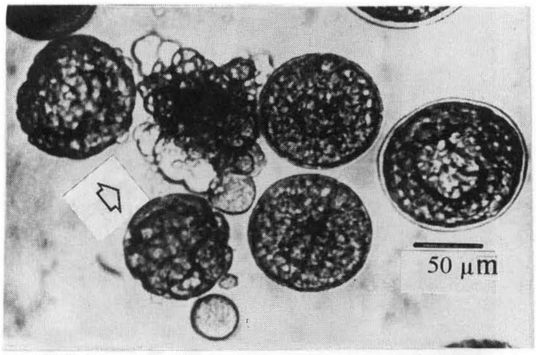

Fig. 5. Cytolysis of embryos (arrow) at the blastula stage in cultures from sites 2, 3, 4.

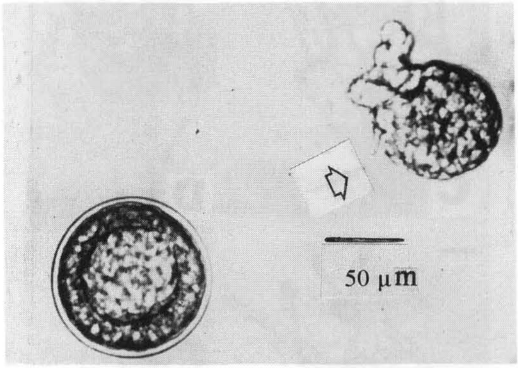

Fig. 6. Exogastrula in cultures from sites 2, 3 (indicated by arrow).

complete their development and metamorphosis.

In the control culture 30–35 day old larvae had developed 8 arms, two rows of epaulettes, and amniotic cavity in which the rudiment of juvenile was formed (Fig. 2 G). These plutei safely metamorphosed into juveniles (Fig. 2 H).

Discussion

We used to do electrostimulation to release gametes from sea urchin for more than 15 years. If this method has been used carefully for welfare of experimental animals, keeping them wet during this procedure, the animals usually survived after electrostimulation and we could use them several times (Naidenko, 1984).

The largest quantity of eggs (ca. 890,000) obtained from one female was from site 2. It is known that during the entire spawning one female *S. intermedius* can produce hundreds million eggs (Kasyanov et al., 1980), and that hundreds thousand of eggs during one shedding (Naidenko, 1984). In the present study a small number of eggs was released by most females irrespective of place of collection. Low fecundity (ca. 150,000–160,000 eggs) of the control females (site 5) can be explained by the immaturity of the gonads; as to low fecundity of females from polluted sites, it must be due to the depression of their gonads which was shown by morphological and histological evidence (Vashchenko et al., 1992)

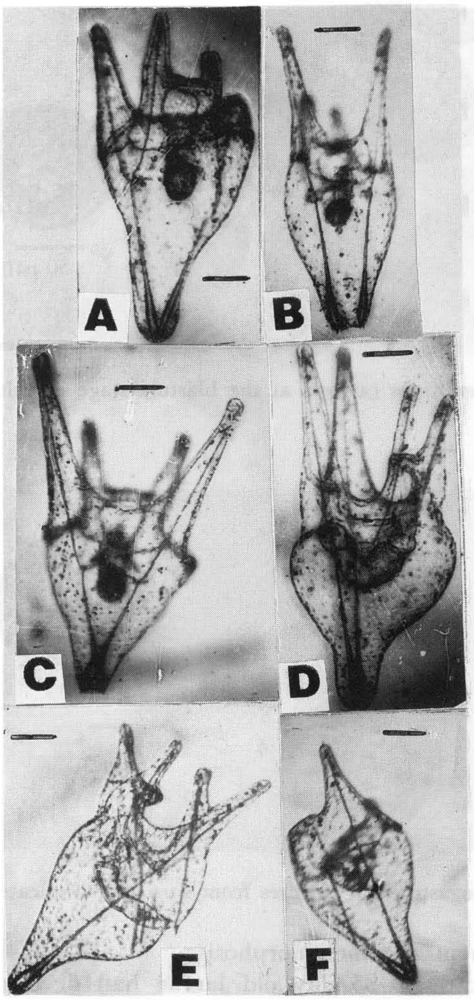

Fig. 7. Abnormal development of 4-armed plutei (sites 2–4): A - body asymmetry, B - divergent basal rods, C - crossed basal rods, D - ugly swollen bodies, E - irreversibly bent arms, F - reduced size arms and smaller body. Scale bar 50 μm.

It should be taken into account that the mortality of planktotrophic larvae of marine invertebrates is usually very high (Kasyanov et al., 1980), and in sea urchin larvae it can reach 99.0 % (Kawamura, 1973). Therefore significant decrease of fecundity can reduce the reproductive potential of sea urchins even in clean areas, to say nothing of polluted ones.

Retardation of the embryogenesis, cytolysis of embryos and a formation of exogastrulaes were observed in the present cultures from polluted sites. These phenonmena were also observed by other studies (Vashchenko et al., 1988; Kobayashi, 1971, 1990) on experimental exposure of gametes and embryos of sea urchin to toxicants; e.g. disturbance of invagination during gastrulation (exogastrula) occured when sea water contains metallic ions in high concentrations (0.046 ppm for $HgCl_2$ and 0.1 ppm for $CuSO_4$; Kobayashi, 1971).

In the present study different abnormalities of development were observed in larval

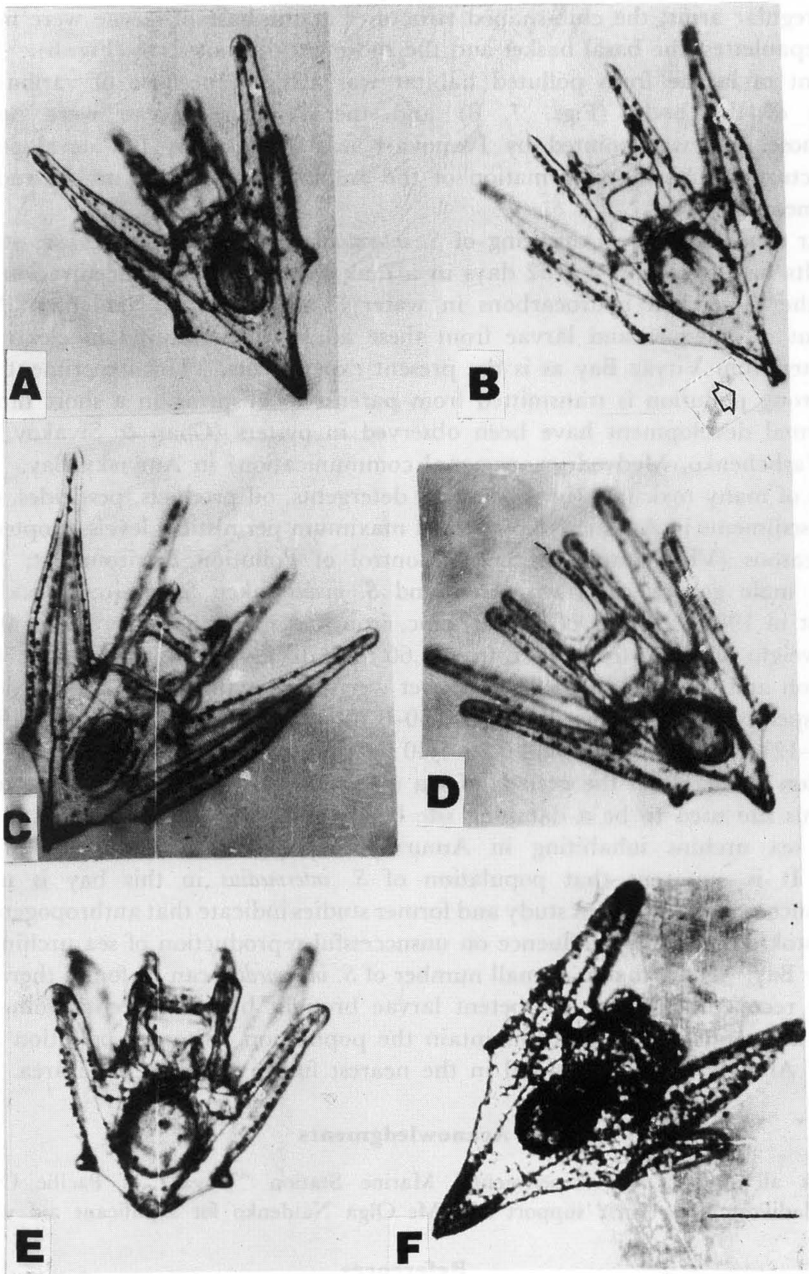

Fig. 8. Abnormal structure of 6- and 8-armed plutei (sites 2, 4). A - abnormally elongated base; basal basket and amniotic cavity are not formed and the lower epaulettes are absent. B - crossed basal rods, the base with a pointed end (indicated by arrow). C - variation in the arm width, abnormal formation of the preoral arms. D - 8-armed pluteus with well-formed arms, the base is elongated, the amniotic cavity is not formed. E - reduced size arms on small body. F - larvae with reduced arms. Scale bar 100 μm.

cultures from the polluted sites. Six and 8-armed abnormal plutei had the elongated bases and the irregular arms; the club-shaped structures at the base of larvae were not resolved, the lower epaulettes, the basal basket and the rudiment did not form (Fig. 8). Subsequent development of larvae from polluted habitat was arrested because of various structural deformities of the body (Figs. 7, 8) and therefore the larvae were not able to metamorphose. As was pointed by Ivanova-Kazas (1978) only full development of all larval structures can lead to formation of the amniotic cavity, sea urchin rudiment and following metamorphosis.

Similar nonviable larval offspring of *S. intermedius* was noted in previous study during which adults had been kept for 52 days in a tank with sublethal concentration (0.04–0.30 mg/l) of the diesel fuel hydrocarbons in water (Vashchenko & Naidenko, 1989). The development of embryos and larvae from these adult were cultured in clean unpolluted filtered water from Vityaz Bay as is the present experiments. This experiment shows that effect of strong pollution is transmitted from parents to offspring in a short time.

Abnormal development have been observed in oysters (Chan & Sivakov, 1991) and scallops (Vashchenko, Medvedeva, personal communication) in Amyrsky Bay.

Levels of many toxicants (heavy metals, detergents, oil products, pesticides, etc.) in the water and sediments in Amursky Bay, exceed maximum permissible levels adopted in Russia in some seasons (Vladivostok Center of Control of Pollution Environment; 1985, 1986, 1988). In male gonads of *S. intermedius* and *S. nudus* taken from sites 2 and 3 in July and August in 1984, contents of copper, zinc, iron and manganese were from 0.30 to 0.80 μg/g wet weight of gonad for copper, from 5.60 to 11.10 μg/g for zinc, from 27.30 to 147.00 μg/g for iron and from 0.20 to 1.80 μg/g wet weight for manganese. In female gonads of these two species content of copper was 0.50–0.70 μg/g wet weight, 26.50–28.50 μg/g for zinc, 25.00–121.00 μg/g for iron and 0.30–1.40 μg/g wet weight for manganese. The biggest concentration of metals in the gonads of sea urchin has been obtained from site 3 (Busev, 1985). This site used to be a damping site before.

Thus sea urchins inhabiting in Amursky Bay produce abnormal and nonviable offspring. It is apparent that population of *S. intermedius* in this bay is not able to reproduce successfully. Present study and former studies indicate that anthropogenic activities of Vladivostok city certainly influence on unsuccessful reproduction of sea urchin inhabiting in Amyrsky Bay. Nevertheless a small number of *S. intermedius* can be found there. Possibly it is partly reconstituted with competent larvae brought by sea currents from other sites, but this is not totally sufficient to maintain the population. Stop of pollution and urgent cleaning of Amursky Bay is required in the nearest future to protect the area.

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