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Invertebrate bioassays with North Sea water samples. I. Structural effects on embryos and larvae of serpulids, oysters and sea urchins

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ABSTRACT: Structural effects of bottom and surface water samples from two dumping grounds in the inner German Bight on the development of three meroplanktonic organisms *(Pomatoceros triqueter:* Polychaeta, *Psammechinus miliaris:* Echinodermata and *Crassostrea gigas,* Mollusca) were investigated. The titaniumdioxide dumping site was sampled immediately after dumping (within the visible waste trail 1 km behind the vessel), and 10 h after dumping. Samples were taken in the sewage sludge deposition area in the intervals between the usual dumping activities, regardless of the exact dumping schedule. The preserved bioassay test organisms were inspected microscopically to count percentages of "normal" larval hatch in test water samples, reference water samples and laboratory aged control water samples (5 to 10 replicates). The relative water quality at various dumping sites was expressed in terms of "net risk"-values (Woelke, 1972) compared to hatching rates observed in the controls. Larval development of *P. triqueter* was significantly suppressed (up to −22 % "net risk") in trail water of the titanium dioxide dump site while the development of sea urchin larvae was still affected in the 10 h surface samples. Hatching of all test organisms in bottom-water samples from the centre of the sewage sludge dump site was affected to different degrees when compared to reference areas about 4 km north or 6 km northwest of the dumping area. The general usefulness of standardized bioassay procedures in pollution monitoring programmes is discussed. The results presented here call for further verification to minimize experimental background variability and to enlarge the catalogue of suitable effects criteria.

INTRODUCTION

Many investigators have tried to estimate the response of benthic populations to man-made environmental changes at dump sites. Commonly used criteria are diversity indices (Pearson, 1975; Rachor, 1977), multivariate analyses of community structures (Eagle et al., 1979) and models of log-normal distribution of species (Gray & Pearson, 1982) which have been used besides many others to describe long-term changes in populations. Other in situ effects monitoring studies include combined laboratory and field investigations to evaluate the individual responses of exposed organisms on a short-term basis. Among the various bioassay methods employed, those using sensitive meroplanktonic life stages of invertebrates such as sea urchins and oysters seem to hold promise in obtaining meaningful biological response data related to water quality in coastal areas. In the past, such "larval bioassays" have been mainly used in laboratory toxicity tests (Greenwood & Brown, 1974; Lönning, 1977; Falk-Petersen, 1979; Karbe et al., 1984). A few of these test procedures have been employed under in-situ conditions

(Okubo & Okubo, 1962; Kobayashi, 1971; Bougis et al., 1979). Unfortunately, the known high variability of biological responses to environmental change has often tended to discourage many investigators from using biological short-term effect studies as a tool in monitoring programmes. Further, the high background noise caused by both the experimental procedure and by the fluctuations of various natural environmental factors at exposure sites is another disturbing problem which needs to be overcome to identify a meaningful signal.

Following the recommendations given by the ICES subgroup "on the feasibility of effects monitoring" (1976, 1978) and by Stebbing et al. (1980), this study aimed to develop a sensitive and standardized test procedure to detect possible biological responses to man-made alterations in the marine environment. The paper describes the initial approach and the first results of bioassay techniques testing North Sea water samples from various dump sites in the laboratory. Special attention is given to detailed methodological descriptions because of the importance of various handling procedures as contributors to experimental background variability. The usefulness of larval bioassay with sea urchins, oysters and polychaetes in future control and effects monitoring studies is discussed.

MATERIAL AND METHODS

Study area

Two dump sites were selected to collect test water samples. They are both located in the inner part of the German Bight (Fig. 1):

Area 1 (54 °1.9′ N – 54° 3′ N / 8° 12′ E – 8°14.1′ E; extension approximately 4.5 km^2; depth 10 to 15 m; muddy silt sediments) was a former sewage sludge dumping ground located in the outer Elbe estuary within an extended zone of muddy sediments. In this area, about 1000 t of sludge from the city of Hamburg were deposited every day since 1962. Dumping ceased in August 1980, when an alternative site was opened in the North Atlantic.

Area 2 (54° 20′ N – 54° 25′ N / 7° 35′ E – 7° 42.5′ E; extension approximately 75 km^2; depth 25 to 28 m; fine sand sediments is the present dumping site for wastes from the titanium dioxide industry near Bremerhaven (FRG). At this site, about 2000 t of sulfuric acid and iron sulfate mixtures were dumped daily. Wastes had been deposited here since 1969.

Several reference test water samples were collected outside the dumping areas during each sampling cruise. In two experimental series (Cruise 1 and 2) additional controls were taken from a standardized "control water", consisting of aged (4 years), filtered (Seitz K 15) and pasteurized (80 °C for 1 h) sea water (31.9 ‰ S; pH 8.2), which was collected near Helgoland.

Field cruises

Data sampled during five cruises provide the basis for this study. They were carried out in January and June 1980 with FRV "Anton Dohrn" (Bundesforschungsanstalt für Fischerei, Hamburg) and RV "Friedrich Heincke" (Biologische Anstalt Helgoland, Helgoland). Water samples were taken from surface and bottom positions at the two dumping grounds and at "reference" sites (Table 1, Fig. 1). Temperature, salinity, pH-

Table 1. Water sampling for bioassay studies in the German Bight (North Sea). Environmental data at sampling positions, sample size and test organisms employed

Experimental conditions	Cruise No. 1	2	3	4	5
Date	10 Jan. 1980	11 Jan. 1980	4 June 1980	5 June 1980	10 June 1980
Sampling station No. (see Fig. 1)	1–4	5–8	9–23	24–30	31–35
Range of temperature (°C)	5.3–6.1	3.2–4.5	9.1–13.4	9.5–11.2	14.1–15.0
Range of salinity (S ‰)	32.7–33.4	27.8–31.2	27.6–30.2	28.9–29.8	28.5–29.6
Range of pH	–	–	8.24–8.68	8.25–8.43	8.59–8.75
Range of oxygen (% saturation)	–	95–96	82–109	74–114	120–136
Tide	ebb (stat. 1–3) next ebb (stat. 4)	flood	ebb (stat. 9–16) flood (stat. 17–20) ebb (stat. 21–23)	flood (stat. 24–25) ebb (stat. 26–30)	ebb
Wind (Beaufort)	E/ENE 3	ENE 3	N 2–3/0	O/NE 1	NE 1
Dumping activities at sampling time	sampling while dumping vessel active	last dumping activity not known	last dumping activity not known	sampling while dumping vessel active	sampling 10 h after last dumping
No. of water samples tested	4 (surface) 2 (bottom)	1 (surface) 4 (bottom)	15 (bottom)	8 (bottom)	5 (surface)
Age of water samples at incubation time (h)	2.5–12.5	3.3–4.5	6.7–17.5	0.5–7.2	4.7–8.1
Test organism employed	*Pomatoceros triqueter*	*Pomatoceros triqueter*	*Psammechinus miliaris*	*Crassostrea gigas*	*Psammechinus miliaris*

value and oxygen content were determined at each sampling point. Water current (tide) and wind force were recorded. The status of the dumping activity was noted in relation to sampling time, if known.

During Cruise 1 in January 1980 (Stations 1 to 4; Fig. 1) two surface water samples were collected directly behind the dumping vessel, at a distance of about 1 km, out of the visible waste water trail. According to information provided by the DHI (Deutsches Hydrographisches Institut, Hamburg) these samples contained up to 17.9 mg $Fe^{3+} \cdot l^{-}$ sea water as precipitated ferric oxide hydrate and had to be filtered prior to the beginning of the incubation trials (folded paper filters; Schleicher & Schuell, type 595 1/2). Two further water samples were taken at a marginal surface and bottom position of the dumping area where no obvious indication of immediate pollution during the

dumping activity could be found. A reference position (surface and 30 m depth) was selected near Helgoland at a distance of 40 km from the dumping area. All samples of this cruise were collected during two consecutive ebb tides within 10 h, winds E/ENE 3 (Beaufort).

During Cruise 2 in January 1980 (Stations 5 to 8; Fig. 1) three bottom water samples were collected in the centre of the dump site of sewage sludge. A reference sample was

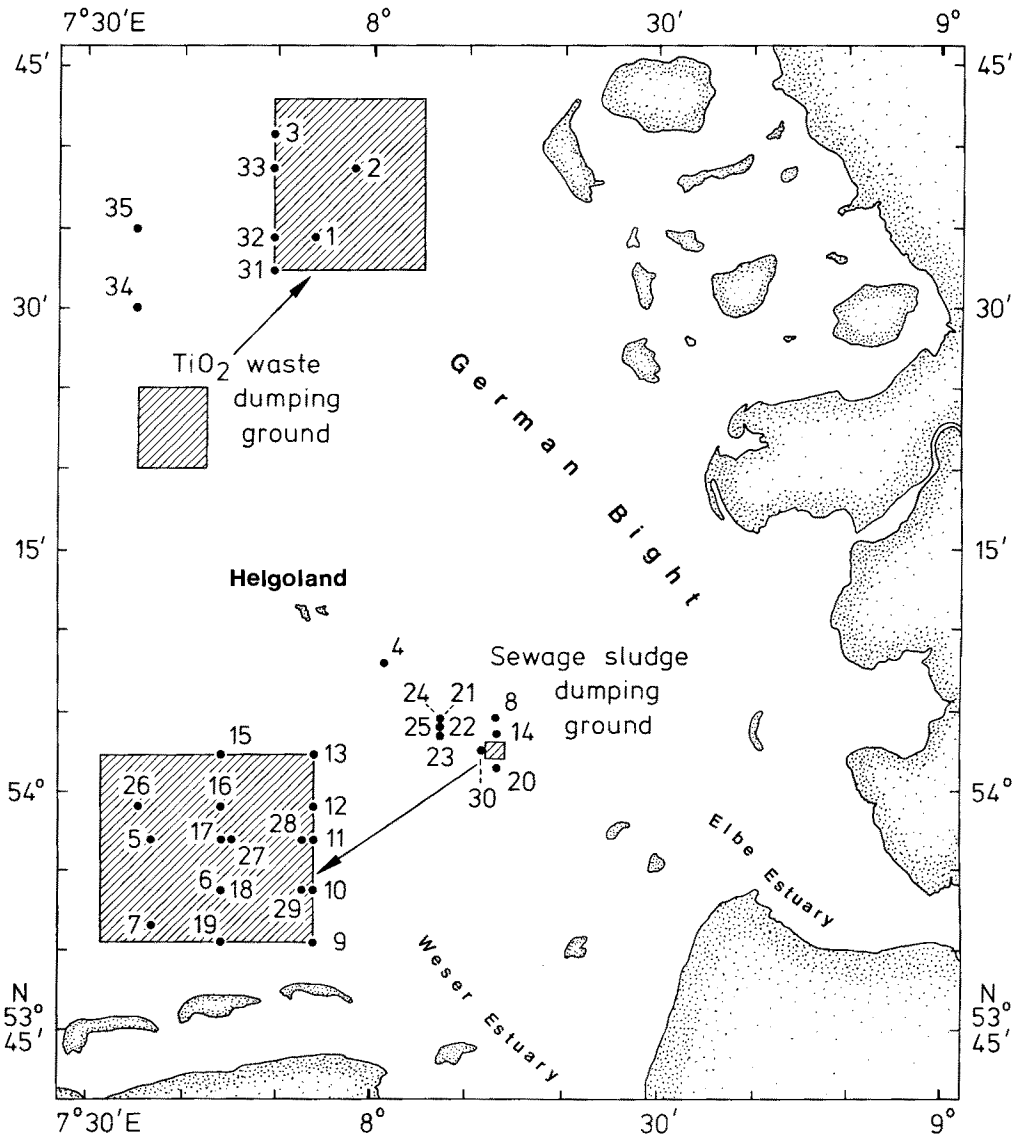


Fig. 1. Position of the two North Sea dumping grounds selected as test areas for bioassay studies; sampling sites and numbers of stations sampled during five cruises in 1980. Environmental data are listed in Table 1

taken from a position about 4 km N of the dumping area. The dumping activity immediately prior to sampling was not known. All samples of this cruise were taken during one flood tide within 1.2 h, wind strength ENE 3.

During Cruise 3 in June 1980 (Stations 9 to 23; Fig. 1) 12 bottom water samples in and around the area of the sewage sludge deposition site were collected. Reference stations were selected from a position about 6 km WNW of the dumping ground. Sampling began at ebb tide. All 15 samples were taken within 11 h. Again, the time lapse between the latest dumping and sampling was not known. North winds of 2 to 3 at the beginning of sampling calmed down to almost 0 when the reference positions had been reached.

During Cruise 4 in June 1980 (Stations 24 to 30; Fig. 1) 6 bottom water samples from the sewage sludge dumping area were collected and compared with water samples from two reference positions 6 km WNW of the dumping ground. These samples were taken within 7 h at flood tide and calm wind conditions (0 to NE 1) (Fig. 1). Station 26 was sampled twice within 20 min. During the sampling period the dumping vessel was in sight.

During Cruise 5 in June 1980 (Stations 31 to 35; Fig. 1) 5 surface sampling positions were selected along a SN transect marginal to the TiO_2 dump site. These samples were taken approximately 10 to 13 h after the last dumping. At that time the waste trail was still visible in surface water in the western parts of the dumping area. No visible precipitations of ferric oxide hydrate were found in filter residues (folded paper filters, Schleicher & Schuell, type 595 1/2). No analysis of iron compounds in the samples was performed. Starting at Station 31, the samples were collected within 3 to 4 h at ebb tide and calm wind conditions (NE 1).

The time lapse between sample collection and beginning of the incubation trials varied slightly between cruises (Table 1).

Sampling methods

"Meteor"-samplers were used to collected 3.5 l water samples from the surface (1 m depth). Bottom water samples (0.3 m distance from the sediment) were taken by a modified specimen of this sampler type. Sea water was filtered immediately after sampling (folded paper filters, Schleicher & Schuell, type 595 1/2) and kept in 1-l glass bottles at 4 °C until the beginning of bioassays (0.5 to 17 h).

Test organisms

Test species used were *Pomatoceros triqueter* (Cruises 1 and 2, January 1980), *Psammechinus miliaris* (Cruises 3 and 5, June 1980) and *Crassostrea gigas* (Cruise 4, June 1980). The polychaetes had been collected from subtidal rocky areas at Helgoland Island, one to two days prior to the experiments. Sexually mature or premature sea urchins from coastal waters near the Island of Sylt (FRG) had been conditioned for extended periods in the laboratory.

Bioassay procedure

The procedures are described in detail because of their importance in minimizing the experimental background noise.

Preparation of test organisms and fertilization

Artificial spawning of *Psammechinus miliaris* was achieved by KCl-injection (1 ml KCl 0.5 m), while opening the shell of mature specimens of oysters and serpulids (Klöckner, 1978) yielded sufficient gametes. Eggs of all test species were collected in 10° to 15 °C filtered sea water and were used within two hours after preparation. Debris was removed by sieving the eggs through a 150 µm gauze. Eggs of all species settled within minutes, allowing repeated washing and decanting (2 to 3 times). Cleaning and fertilization procedures were performed in "control water" (see above).

Vital sea urchin sperm was collected "dry" and could be stored for several hours in covered petri dishes at 4 °C. Sperm of the other species was used directly. Immediately prior to fertilization, sperm was diluted with filtered sea water. Egg density was determined in a plankton counting chamber (35 mm diameter, marked bottom grid of 1 mm) under the inverse microscope. *Psammechinus miliaris* sperm was carefully dosed (ratio about 50 sperms per egg). Egg density during fertilization was kept at 10^4 eggs per ml. This procedure helped largely to avoid "polyspermy" (< 3 %). Whenever possible a single female and male of the respective test species were used.

One to two hours after fertilization, embryos of 2- to 8-cell stages were transferred to small incubation boxes containing test water subsamples using a calibrated pipette. Egg batches of sea urchins and oysters resulting in less than 80 % normally developed cleavage stages were discarded. Only few fully mature specimens of *Pomatoceros triqueter* were available, forcing us to accept lower fertilization rates (< 50 %) in the January 1980 trials.

Incubation

Pomatoceros triqueter embryos were incubated in glass vessels (1000 eggs in 10 ml test water; 7 replicates per sample). Sea urchins and oysters were incubated in polystyrene boxes (3000 eggs in 30 ml test water; 5 replicates). Reference water samples were tested in 10 replicates.

Test boxes with eggs were kept in trays [dark; constant temperature room, incubation temperatures: 20 ± 1 °C (*Pomatoceros triqueter*), 24 ± 1 °C (sea urchins and oysters)].

Developmental stages were identified during incubation time at 2 to 6 h intervals. Free swimming endotrophic larval stages in the control series were reached after 24 to 32 h of incubation: the echinopluteus (*Psammechinus miliaris*), the D-shaped veliger (*Crassostrea gigas*) and the trochophora (*Pomatoceros triqueter*). When certain developmental stages occurred in the controls, all test trial replicates were considered as sufficiently advanced in their development for evaluation of the series. Organisms were preserved in 4 % formaldehyde, buffered with borate at pH 8.0.

Effects criteria

Subsamples of about 1000 (serpulids) or 400 to 500 (sea urchins and oysters) developmental stages were taken from each replicate. Total number of organisms in

each subsample was counted under the inverse microscope (magnification 125 x) using the plankton counting chamber, described above.

The ratio of "normally" developed larvae was expressed as the percentage of all developmental stages obtained, including the unfertilized eggs as well as the retarded and deformed specimens (sea urchins and serpulids). In the case of oysters the percentage of "normal" larvae, however, was determined from the total number of larvae alone (including abnormal larvae, excluding other developmental stages). Criteria used for effects evaluation followed species specific morphological characteristics. Catagories of deviation from the "normal" development were noted. Larval normality was defined for each species as follows:

Pomatoceros triqueter: 24 h old normal trochophora larvae showed the following features: (1) Prototrochal cilia, (2) ectodermal pigment cells, (3) complete gut structures.

Crassostrea gigas: 24 h old normal D-shaped veliger larvae exhibited (1) a fully developed, symmetrical shell and (2) complete soft structures including the velum. Any retardations resulting in incomplete soft parts or deformed shells (asymmetrical shape, concave D-line, carved shells) were considered abnormal.

Psammechinus miliaris: Normal pluteus larvae obtained after (a) 24 h and (b) 32 h showed the following features:

(a) The 24 h old endotrophic pluteus possessed short post oral arms; oral arms were not yet developed. Criteria of normality were: (1) Complete outer larval contours (body, post oral arms, oral lobe), (2) Complete set of skeletal rods (body rods, transversal rods, post oral rods, oral rods not exceeding the oral lobe), (3) complete gut structures (mouth, stomach, intestine). Plutei with deformed or missing parts of the body or skeletal apparatus (missing or doubled rods) were considered abnormal.

(b) After 32 h of incubation an advanced, 4-armed almost ectotrophic echinopluteus was formed, possessing one pair of long post oral arms and one pair of shorter oral arms (Fig. 2b). Criteria of normality here were: (1) Complete outer contours (body, post oral and oral arms, the latter at minimum budding in the oral lobe), (2) complete set of skeletal rods [set as described for (a), but oral rods exceeding the mouth field], (3) complete gut structures [as decribed for (a)]. Plutei with deformed or missing parts of the body (e.g. unequal length of post oral and oral arms), or with skeletal abnormalities (missing, twisted, doubled or apically "crossed" rods were considered abnormal; see Figs 2c–h).

When incubating sea urchin eggs for 32 h, plutei of both control and test series showed varying numbers of individuals with apically "crossed" body rods (Fig. 2h). Regardless of the biological meaning of this deformation, in one experimental series (Cruise 3) counting of the total sample size (all replicates) was repeated by modifying the criteria of normal development: When the larva was placed in ventral position, post oral arms had to be equal in length, exceeding the oral lobe; apically "crossed" body rods, further, were neglected. Compared to the first counting this procedure resulted in a significantly lower deformation rate in water samples of the reference (reduction from 16.8 % to 10.3 % deformed larvae). Obtaining nearly identical "net risk"-values (see below) when applying both counting procedures, experimental background variability could be reduced and reproducibility improved by the second handling (see results depicted in Fig. 4a).

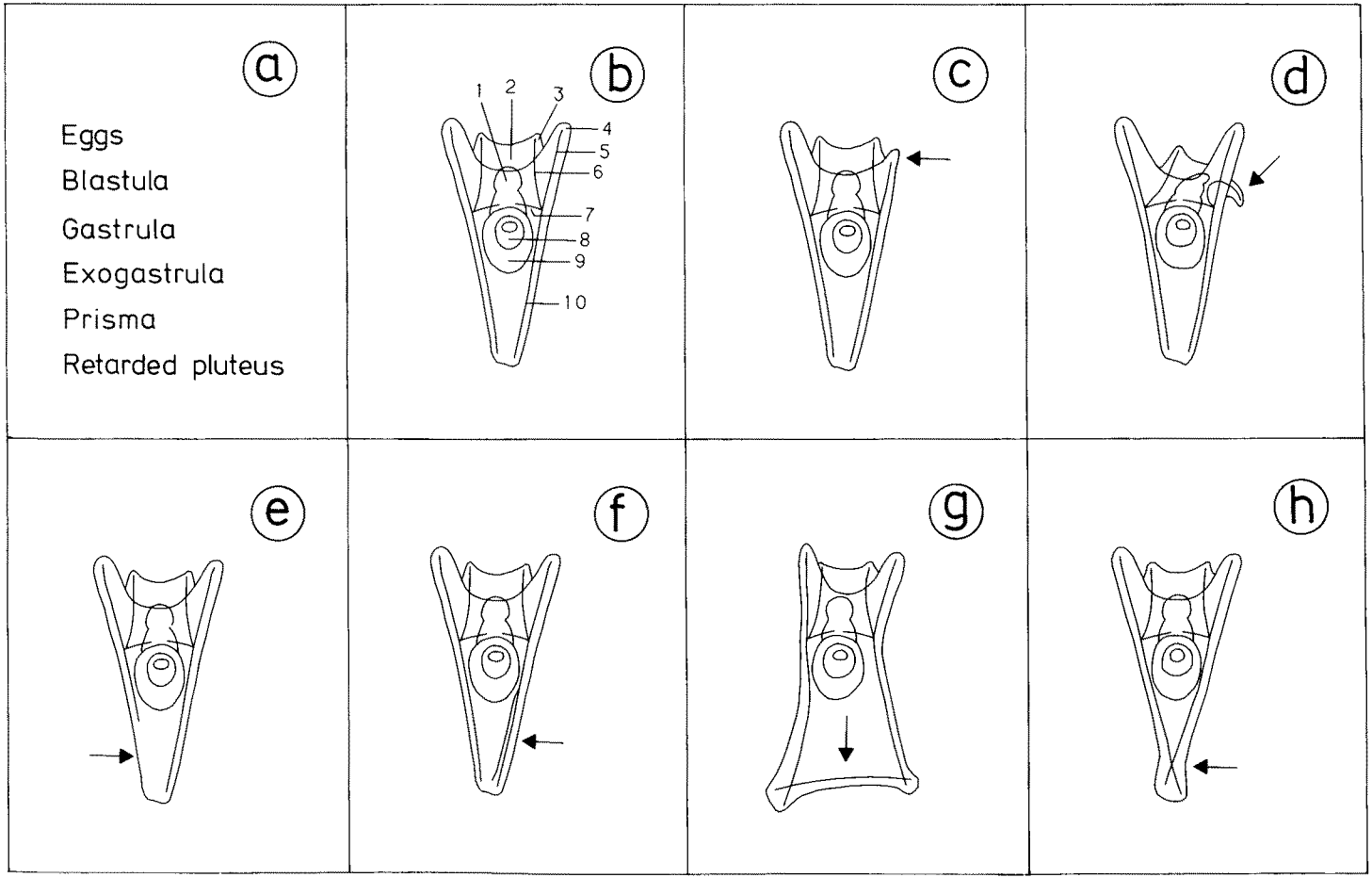


Fig. 2. Illustration of structural criteria to define normal and abnormal larval development of *Psammechinus miliaris* at the stage of the 4-armed echinopluteus, incubated for 32 h at 24 °C. "Normal" development: echinopluteus as depicted in (b). "Abnormal" development: (a) various deformed or retarded developmental stages from egg to 2-armed echinopluteus. (c–h) various types of deformed echinopluteus larvae: (c) unequal length of post oral arms. (d) twisted right oral rod. (e) parts of right body rod missing. (f) left body rod doubled. (g) additional crossbarred body rod. (h) apically "crossed" body rods. Numbers in (b) indicate: 1 mouth; 2 oral lobe; 3 oral arm; 4 post oral arm; 5 post oral rod; 6 oral rod; 7 transverse rod; 8 intestine and anus; 9 stomach; 10 body rod. The echinopluteus is shown from ventral position (original size about 350 µm). Consult detailed description under "Material and Methods"

Data analysis

Differences in the relative rate of normal development as observed in each test series (water samples from the dumping grounds, from reference areas and from control water) were described by calculating the "net risk" (NR) of test water samples, a term introduced by Woelke (1972). This procedure made correction of the raw hatching data possible, as it standardizes the varying abnormality rates in controls resulting from the quality of test organisms and experimental handling:

$$NR\ (\%) = (N_T - N_R)\ 100/N_R$$

NR = "net-risk"-value of a test water sample (%); N_T = mean relative number of normally developed larvae in replicates of test water sample; N_R = mean relative number of normally developed larvae in replicates of reference water sample. N_T and N_R refer to the rates of normal larvae (raw data) on the basis of the total number of incubated eggs (sea urchins, serpulids) or on the basis of the total number of normal and abnormal larvae hatched together (oysters).

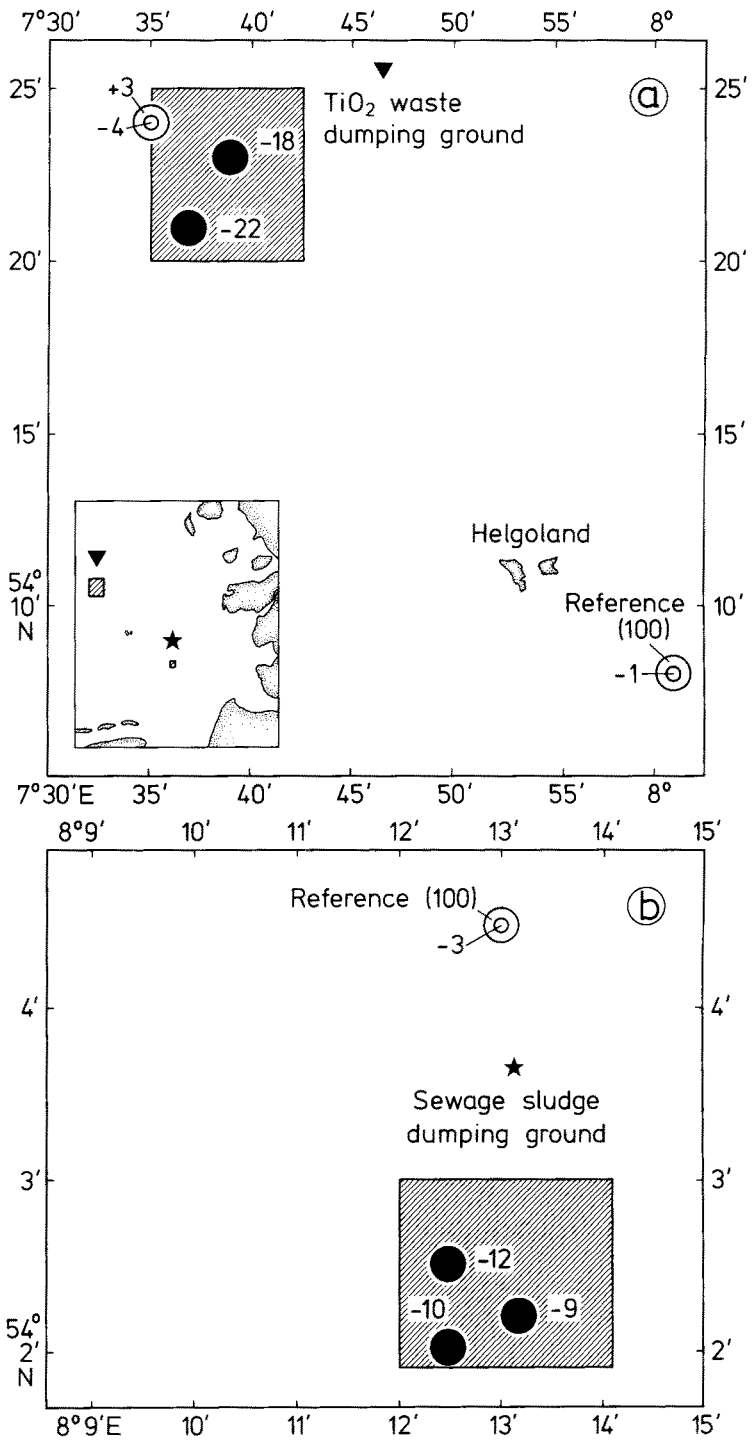
Differences between relative hatching rates were tested by using oneway analyses of variance, after transformation of raw data into arc sin $\sqrt{P}$ (Sokal & Rohlf, 1969). "Net risk"-values below -5 % were generally found to be significant at the $p < 0.05$-level. In few cases only were positive differences between test water and reference water samples observed.

RESULTS

Polychaete incubation trials

Figure 3a depicts the results obtained during Cruise 1 (January 1980) incubation experiments with *Pomatoceros triqueter* using water samples collected at the dump site for titanium dioxide wastes. The mean rate or normally developed trochophorae in the reference surface sample was found to be 34.0 ± 2.6 % of the total number of incubated eggs. This value served as the basis to estimate the "net risk"-values for the test water samples. Significantly lower hatching rates were obtained for the two central surface positions taken immediately after dumping (27.9 ± 2.4 % and 26.5 ± 2.5 % normal larvae) with "net risk"-values of −18 and −22 %. These values clearly indicated the poor water quality of the directly polluted water masses at the dumping site. The two samples taken at the marginal surface and bottom position, however, did not show significantly different results compared to reference water ("net risk"-values −4 and +3 %) and laboratory "control water" (−5%) tests.

During Cruise 2 in January 1980 (Fig. 3b) incubation trials with *Pomatoceros triqueter* in reference bottom water (4 km N of the sewage dump site) yielded 38.8 ± 0.8 % normal larvae (based on total number of the incubated eggs). No significant differences of "net risk"-values were found between reference surface water (−3 %) and laboratory "control water" (+1 %). Larval development was significantly affected, however, in the three bottom water samples taken at central positions of the dumping ground. Percentage of normal larvae obtained here were 34.1 ± 1.2 %, 35.2 ± 0.3 % and 34.7 ± 1.1 % respectively, resulting in "net risk"-values of −9 to −12 %.


7°30'
35'
40'
45'
50'
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20'
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54° 10' N
+3
-4
-18
-22
TiO2 waste dumping ground
a
Helgoland
Reference (100)
-1
7°30'E
8°9'
10'
11'
12'
13'
14'
15'
4'
3'
54° 2' N
8°9'E
Reference (100)
-3
b
Sewage sludge dumping ground
-12
-10
-9

Sea urchin incubation trials

Figure 4a depicts the results obtained during Cruise 3 (June 1980) incubation experiments using *Psammechinus miliaris* as test organism. Mean hatching rates at the reference positions resulted in 66.3 ± 1.8 % normally developed larvae. Compared to the reference, significantly lower hatching rates were found in all test water samples (range of "net risk"-values: −4 to −26 %). The lowest values were observed in the water samples at Station 11 (Fig. 1) resulting in mean rates of normal larvae of 49.4 ± 3.5 %. Samples from three other stations within the dump site area (Stations 12, 13, 18) showed 53.0 ± 1.3 %, 51.2 ± 5.6 % and 54.8 ± 1.8 % normal development. "Net risk"-values calculated from these data were −20, −23 and −17 % respectively.

A first processing of the Cruise 3 samples gave a significantly higher rate of deformations, thereby obtaining lower rates of normally hatched larvae in reference water samples (59.8 ± 1.5 %; application of different effects criteria: consult detailed description in "Material and Methods"). Also in test water samples from dump site positions (Stations 12, 13, 18; Fig. 1) these values were reduced (47.5 ± 1.6 %, 45.9 ± 3.2 % and 48.4 ± 1.5 %). The relative differences between both counting results, however, did not significantly change the estimated "net risk"-values at any position of this cruise.

The results obtained with *Psammechinus miliaris* incubation trials during Cruise 5 (June 1980) are shown in Figure 4b. In water samples from the reference area (Station 35, about 20 km N of the TiO_2 waste dump site; Fig. 1) 61.5 ± 1.2 % normally developed 2-armed plutei were obtained. At a marginal SW position of the dumping area (Station 31) hatching rates only reached 53.9 ± 1.8 %. Compared to the reference these data presented a high "net risk"-value of −12 %. Significantly low hatching rates were also obtained for two other sampling positions (Stations 32 and 34: both 59.4 ± 1.7 %), the latter position being situated about 9 km N of the dumping area where no visible trail of the titanium dioxide wastes had been detected at sampling time (about 12 h after the last dumping activity). Calculated "net risk"-values for both positions were approximately −3 %.

Oyster incubation trials

During Cruise 4 (June 1980) we tested effects of bottom water samples from in and around the sewage sludge area on the development of *Crassostrea gigas* eggs. Two different analyses of the counting results are presented here (Fig. 5a and b).

Based on the total number of initially incubated eggs, including those which were not fertilized, a mean hatching rate of 65.3 ± 3.3 % of normally developed larvae was found in the samples from the reference stations. Corresponding "net risk"-values at the two sampling stations from the western part of the dump site ranged between −1 %

Fig. 3. (a) Bioassay series with *Pomatoceros triqueter* from 10 January 1980 in surface water samples from the TiO_2 waste dumping ground and (b) from 11 January 1980 in bottom water samples from the dumping ground of sewage sludge. Numbers indicate "net risk"-values of "normal" larval hatch (referred to the stage of the trochophora) compared with reference sites (index of 100 % hatch). Filled circles: Negative difference of relative normal hatch to the reference significant at $p < 0.05$. Small open circles: Results obtained at reference position in bottom water (a) or surface water (b)

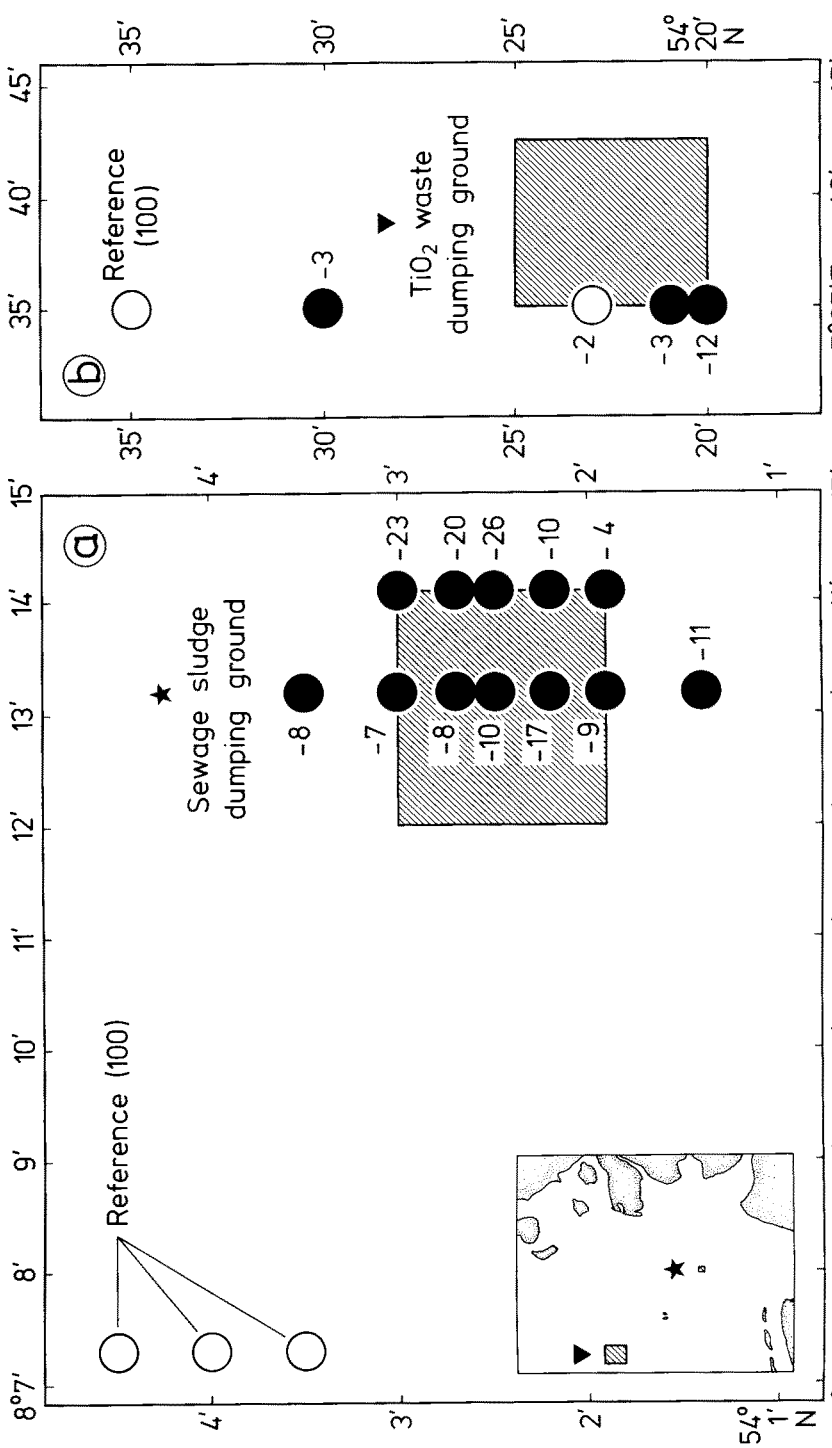

Fig. 4. (a) Bioassay series with *Psammechinus miliaris* from 4 June 1980 in bottom water samples from the dumping ground of sewage sludge, and (b) from 10 June 1980 in surface water samples from the TiO_2 waste dumping ground. Negative numbers indicate "net risk"-values of "normal" larval hatch [referred to (a) the 4-armed echinopluteus or (b) the 2-armed echinopluteus] compared with reference sites (index of 100 % hatch). Symbols used as in Figure 3

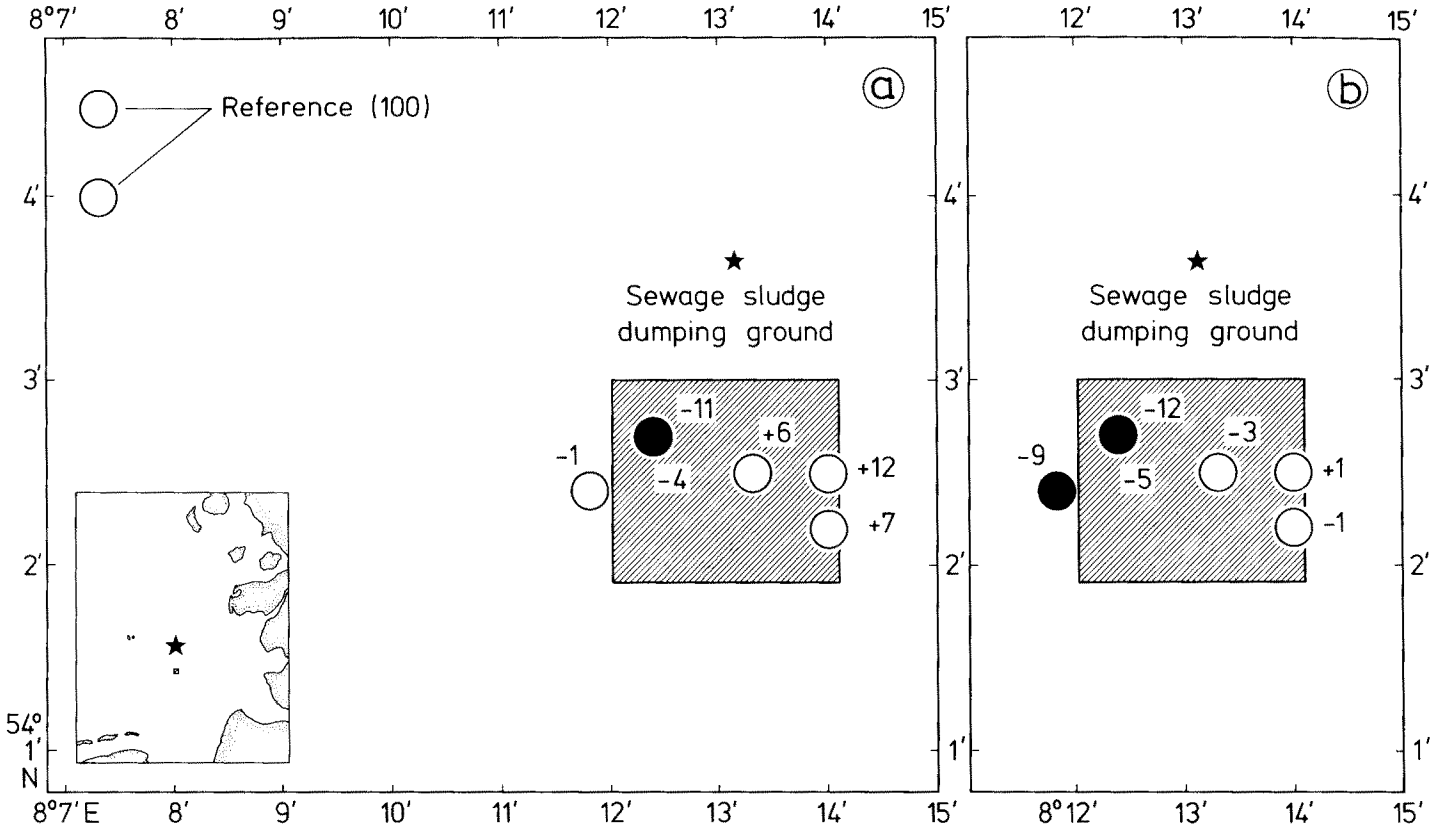

Fig. 5. Bioassay series with *Crassostrea gigas* from 5 June 1980 in bottom water samples from the dumping ground of sewage sludge. Numbers indicate "net risk"-values of normal larval hatch (referred to the "D"-larva) compared with the reference water samples (index of 100 % hatch). (a) Calculation of "net risk" is based on the total number of incubated eggs, or (b) on the total number of hatched larvae. Different results were found at one station which was sampled twice within 20 min (−12 and −5 % "net risk"). Symbols used as in Figure 3

(64.5 ± 5.2 % normal hatch) and −11 % (58.6 ± 3.6 % normal hatch; Station 26 upper value), while for three eastern positions of the test area these "net risk"-values deviated positively from the reference (+6 to +12 %) corresponding to 68.9 ± 5.3 % and 69.9 ± 6.0 % normal larvae (Fig. 5a). The second evaluation of the counting results was based on the total number of hatched larvae, neglecting other stages (Fig. 5b). Normal larvae in reference samples amounted to 91.6 ± 1.8 %. This basis of calculation resulted in generally higher negative "net risk"-values at the two westerly sampling stations (−5 to −12 %) corresponding to 86.8 ± 4.3 % and 80.5 ± 6.0 % normal hatch. Larval hatch in sample stations of the eastern part of the dump site, however, was close to values at the reference stations (−3 to +1 %) corresponding to 88.7 ± 3.2 % and 91.2 ± 5.4 % normal hatch. The different calculation procedures indicate that the experimental background noise can be slightly reduced when excluding unfertilized eggs from data evaluation in the case of oyster incubation experiments.

DISCUSSION

At the 1979 ICES workshop on problems of pollution monitoring, Stebbing et al. (1980) postulated principal requirements within bioassay procedures in biological monitoring programmes concerning the choice of test organisms (e.g. ecological relevance, sensitivity, feasibility of culturing, availability throughout seasons, genetic homogeneity) and the practicability of bioassay techniques (e.g. simplicity of procedures, time and cost efficiency, statistical accuracy).

Among the recently described techniques, preference was given to larval bioassays with sea urchins and oysters at these conferences. Based on initial trials with sea urchin embryos published by Kobayashi (1971) and with oyster larvae employed by Woelke (1972) the applicability of such procedures to characterize water quality was tested at several places in the world. All results so far presented, however, demonstrate the need for further verification of the experimental approach, to distinguish better between inherent and experimental error.

Several subsequent applications of oyster larval bioassay procedures have been worked out in North America: Pacific oyster embryos were used to assess the quality of receiving water prior to and after reduced emission of sulfite pulpmill effluents in the Puget Sound (Washington State) between 1972 and 1975 (Cardwell et al., 1977). These authors demonstrated that the extent of receiving water toxicity to oyster embryos and larvae was reduced from 30 km^2 to about 1 km^2. Other test series carried out in the Pacific (1979, Ladysmith Harbour, British Columbia), using early life history stages of *Crassostrea gigas*, showed similar applicabilities, indicating a clear gradient of water quality deterioration. This trend was followed by using surface water samples from the mouth of the harbour, where water quality was best, to the inner part of the bay, where it was poorest (Bourne et al., 1981). However, a significant gradient could only be demonstrated during summer months. Nelson et al. (1983) used *C. virginica* larvae to determine the percentage of abnormally developed larvae in test water samples while monitoring the water quality on a dredge-spoil dump site near Black Rock Harbor (Bridgeport, Connecticut). They found a significantly increased deformation rate in test water samples taken during active dumping while those taken one month after the last

dumping still showed some negative effects compared to the results obtained prior to the beginning of the dumping activities.

Bougis et al. (1979) provided another example to quantify biological effects in degraded environments. This time sea urchin eggs and larvae (*Paracentrotus lividus*) were exposed to sea water samples from different locations of the Mediterranean Sea. These water samples were additionally contaminated with various copper concentrations. The change in LC50-values for copper tolerance in sea water samples was compared to copper toxicity in standard water and interpreted as a biological effect of the environmental conditions at sampling sites. The authors, therefore, aimed at the determination of a relative sensitivity. We have not attempted to employ such procedures, since it seems to limit information through introduction of an additional variable. Bougis (1967), and later Greenwood (1980), proposed a technique in which a meristic character (the body size of sea urchin larvae) can be used as effects criterion. So far, however, this method has been employed at a few sampling stations in South African waters, and the value of this criterion needs further verification.

A recent approach undertaken by Bay et al. (1983) seems to hold promise as a simplified tool in larval bioassay procedure with sea urchins. This technique is based on a physiological effects criterion: the inhibition of the "echinochrome"-synthesis during larval development. Pigment synthesis in *Strongylocentrotus purpuratus* larvae was suppressed in water samples from a Californian sewage sludge dumping area (Oshida et al., 1982). Presently, in our laboratory, studies are under way to investigate the utility of this criterion, using European sea urchin species.

A comprehensive summary of the available literature on ecotoxicological tests with echinoderms has recently been presented by Kobayashi (1984). Further, Stebbing & Brown (1984) provided examples on the use of coelenterates as potential test organisms. The results reported are encouraging enough to warrant the use of bioassays in monitoring programmes. They indicate that under certain local conditions (i.e. restricted dumpsite areas, discharges outflows or estuaries) the embryonic and larval stages of sensitive invertebrates are useful as indicators of trends in water quality changes in affected areas.

One of the improved procedure was extensively applied by authorities of the British Ministry of Agriculture, Fisheries and Food (MAFF) in routine monitoring surveys of British marine coastal waters using oyster (*Crassostrea gigas)* larvae (Lloyd & Thain, 1981). In a further study (Thain & Watts, 1984), it was shown that the biological water quality associated with the development of an unusual plankton bloom (*Gyrodinium aueolum*) could be monitored by using an oyster embryo bioassay. It was even possible to demonstrate the duration of the deleterious effect of this bloom up to four weeks after its collapse. The authors used the "net risk"-values as criterion (Woelke, 1972). The evaluation method employed, therefore, appears to be a valuable tool to characterize suitable changes in relative water quality.

Our results presented here also indicate a general usefulness of the early life cycle stages of all three species investigated for studies in the field of biological effects monitoring. The criteria employed to quantify morphological effects on development seemed to be sufficiently sensitive to detect relative differences in North Sea water masses. The available data indicate that similar deviations from normality can be observed in test water samples from both dumping grounds. The procedure, therefore, can be considered as non-specific. This is not discouraging, since the present study does

not aim at identifying cause-relationships for specific pollutional agents, but concentrates its efforts on finding integrated responses of individuals which reflect the overall relative environmental stress imposed on biota, in exposed and non-exposed areas.

Laboratory toxicity experiments with our test organisms have shown that in situ effects may be compared with the most sensitive reactions to the lowest effective heavy metal concentrations experimentally applied: in a screening test we observed abnormal development of *Pomatoceros triqueter* as well as *Psammechinus miliaris* (24 h incubation at 20° or 24 °C) at initial mercury concentrations (Hg^{2+} as $HgCl_2$) of 5 µg Hg per l. Similar ranges of sensitivity to mercury, copper and other metals during embryogenesis of different species of sea urchins and oysters tested are reported in the literature (Waterman, 1937; Okubo & Okubo, 1962; Calabrese et al., 1973; Kobayashi, 1981; Martin et al., 1981).

Provided that both field recruitment and cultivation facilities enable a sufficient supply of sexually mature test organisms, the techniques described here are in principle simple to carry out and easy to evaluate. As demonstrated, the procedures can be largely standardized. Especially the use of sea urchin larvae at an advanced pluteus stage provided reliable data on differences between water masses, thereby determining the relative water quality in adjacent areas (Fig. 4a). However, the variability found within subsamples of test water was substantial (coefficients of variance up to about 11 %), indicating that a large number of replicates as well as high numbers of test specimens within each replicate were required to proceed with meaningful statistical treatment and to identify significant differences between sampling stations. The evaluation of a test series, therefore, was time consuming, since an estimation of the hatching rates of sea urchin larvae by a single subsample of 500 developmental stages took about 3 h, resulting in a delayed information of 6 to 8 weeks, when a series of 15 test water samples in replicates of 5 subsamples was required. The large number of test specimens required to identify differences between effects of water samples from different areas is still unsatisfying, because of the time-consuming efforts necessary.

Our laboratory in Hamburg did not provide facilities for oyster conditioning, and only one field series was conducted with *Crassostrea gigas* (Fig. 5). Difficulties were met with when counting developmental stages of this species since unfertilized eggs and retarded specimens often dissolved before preservation time and could not be quantified correctly by fragments. Further, no sufficient catalogue of effects criteria was found to classify abnormal development of the veliger, resulting in high variabilities (coefficients of variance within subsamples of up to about 13 %).

Pomatoceros triqueter larvae are sensitive and easily available test organisms which mature sexually especially in summer and autumn, and even throughout the whole year. Variability in the counting of normally developed trochophorae was similar to that of pluteus larvae (coefficients of variance within subsamples up to about 10 %). The test species, however, appears to be of limited use in extended bioassay series because the number of eggs per female does not exceed 50 000 whereas sea urchins and oysters may yield one to several million mature eggs per female. Considering methodological and biological aspects of the bioassay procedures employed in this study, sea urchins are ranked highest as test organisms.

The results of our experiments have to be interpreted with caution since biological effects generally may not be related to specific pollution factors but probably include an

integrated response to environmental stress. Only in two experimental series could biological effects be associated formally to hydrographical factors: temperature and pH-values were both correlated positively to the hatch of oyster larvae ($p < 0.05$; Fig. 5). No definite conclusion could be drawn either from a formal negative correlation ($p < 0.01$) between hatching of sea urchin larvae and the age of the water samples, since highest hatching rates occurred both in oldest (17.5 h) and freshest (6.7 h) water samples collected during Cruise 3 (Fig. 4a).

Although in many test water samples negative effects on larval development were observed when data were compared to "clean" references and laboratory "control water", a direct association of effects with stress caused by dumping activities and defined pollutants alone was not attempted, because the number of test series performed so far was limited, and parallel chemical background data analysis on toxicant levels was not performed.

Negative effects on egg incubation of *Pomatoceros triqueter* and *Psammechinus miliaris* have been, nevertheless, clearly demonstrated in those surface water samples taken immediately (Fig. 3a) or 10 to 13 h (Fig. 4b) after the last dumping of titanium dioxide wastes. Our observations on visible persistance and drift of dumped TiO_2-wastes are supported by investigations of the Deutsches Hydrographisches Institut (Weichart, 1975). Probably, calm weather conditions provided bioassay results with evidence of the environmental hazard of these wastes. "Normal" weather conditions with higher water turbulence, however, will probably cause an immediate and high dilution of the wastes dumped, and gradients of pollution within the areas investigated in this study are, therefore, more difficult to identify than those reported in the literature above, where a known source of pollutant affected a relatively stable and enclosed area.

In order to detect pollutional gradients, a further substantial reduction in the experimental background noise is necessary. It is for this reason that we have concentrated on a detailed description of our test procedure. An exact definition of the methods used and the effects criteria employed is an important pre-requisite for conducting repeated bioassay surveys in a dumping area.

The development of new effects criteria which allow a reduction in time and man power for test evaluation is another concern of interest and reflects present activity of our laboratory: standardized larval bioassay series with sea urchins (*Psammechinus miliaris* and *Paracentrotus lividus*) undertaken between 1981 and 1983 used meristic as well as biochemical criteria (body size and "echinochrome"-synthesis of the larvae). The results of these experiments will be presented in a forthcoming paper discussing in detail the applicability of our test procedures developed so far.

If such methods can be developed further to employ simple handling procedures the frequency of sampling during surveys could be increased, thereby producing a more continued observation of water masses which usually change very quickly. Within multidisciplinary surveying programmes (including the chemical monitoring of xenobiotic accumulation in water, sediment and organisms), larval bioassay technique is considered to play a potentially useful role.

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LITERATURE CITED

Bay, S. M., Oshida, P. S. & Jenkins, K. D., 1983. A simple new bioassay based on echinochrome synthesis by larval sea urchins. – Mar. environ. Res. *8,* 29–39.

Bougis, P., 1967. Utilisation des plutéus en écologie expérimentale. – Helgoländer wiss. Meeresunters. *15,* 59–68.

Bougis, P., Corre, M. C. & Etienne, M., 1979. Sea urchin larvae as a tool for assessment of the quality of sea water. – Annls Inst. oceanogr., Paris *55,* 21–26.

Bourne, N., Rogers, H., Mahood, H. & Neil, D., 1981. Water quality study in Lady Smith Harbour, British Columbia. – Can. tech. Rep. Fish. aquat. Sci. *1026,* 1–57.

Calabrese, A., Collier, R. S., Nelson, D. A. & MacInnes, J. R., 1973. The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica.* – Mar. Biol. *18,* 162–166.

Cardwell, R. D., Woelke, C. E., Carr, M. J. & Sanborn, E. W., 1977. Evaluation of the efficacy of sulfite pulp mill pollution abatement using oyster larvae. In: Aquatic toxicology and hazard evaluation. Ed. by F. L. Mayer & J. L. Hamelink. American Society for Testing and Materials, Philadelphia, 281–295.

Eagle, R. A., Hardiman, P. A., Norton, M. G. & Nunny, R. S., 1979. The field assessment of effects of dumping wastes at sea: 4. A survey of the sewage sludge disposal area off Plymouth. – Fish. Res. tech. Rep. *50,* 1–28.

Falk-Petersen, I.-B., 1979. Toxic effects of aqueous extracts of Ekofisk crude oil, crude oil fractions, and commercial oil products on the development of sea urchin eggs. – Sarsia *64,* 161–169.

Gray, J. S. & Pearson, T. H., 1982. Objective selection of sensitive species indicative of pollution-induced change in benthic communities. I. Comparative methodology. – Mar. Ecol. *9,* 111–119.

Greenwood, P. J., 1980. Possible uses of the developmental embryology of the sea urchin *Parechinus angulosus* for pollution monitoring in the nearshore marine environment. Ph. D. thesis, Univ. of Cape Town, 334 pp.

Greenwood, P. J., & Brown, A. C., 1974. Effect of ammonium nitrate solutions on fertilization and development of the sea urchin *Parechinus angulosus.* – Zool. Afr. *9,* 205–209.

ICES, 1976. ICES working group on pollution baseline and monitoring studies in the Oslo Commission and ICNAF areas. Report of the sub-group on the feasibility of effects monitoring. – C. M./ICES, *E 44,* 1–36.

ICES, 1978. On the feasibility of effects monitoring. – Coop. Res. Rep. *75,* 1–42.

Karbe, L., Borchardt, T., Dannenberg, R. & Meyer, E., 1984. Ten years of experience using marine and freshwater hydroid bioassays. In: Ecotoxicological testing for the marine environment. Ed. by G. Persoone, E. Jaspers, & C. Claus. Lab. Biol. Res. Aquat. Pollut., State Univ. Ghent, Ghent, *2,* 99–129.

Klöckner, K., 1978. Zur Ökologie von *Pomatoceros triqueter* (Serpulidae, Polychaeta). II. Einflüsse der Temperatur auf Toleranz, Tubusregeneration, Sauerstoffverbrauch und Filtrierleistung. – Helgoländer wiss. Meeresunters. *31,* 257–284.

Kobayashi, N., 1971. Fertilized sea urchin eggs as an indicatory material for marine pollution bioassay, preliminary experiments. – Publs Seto mar. biol. Lab. *18,* 379–406.

Kobayashi, N., 1981. Comparative toxicity of various chemicals, oil extracts and oil dispersant extracts to Canadian and Japanese sea urchin eggs. – Publs Seto mar. biol. Lab. *26,* 123–133.

Kobayashi, N., 1984. Marine ecotoxicological testing with echinoderms. In: Ecotoxicological testing for the marine environment. Ed. by G. Persoone, E. Jaspers, & C. Claus. Lab. Biol. Res. Aquat. Pollut., State Univ. Ghent, Ghent, *1,* 341–405.

Lloyd, R. & Thain, J. E., 1981. The use of the oyster bioassay to monitor coastal water quality. – ICES *WG MPNA 5(1/2),* 1–4.

Lönning, S., 1977. The sea urchin egg as a test object in oil pollution studies. – Rapp. P.-v. Réun. Cons. int. Explor. Mer *171,* 186–188.

Martin, M., Osborne, K. E., Billig, P. & Glickstein, N., 1981. Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvae. – Mar. Pollut. Bull. *12,* 305–308.

Nelson, D., Miller, J., Pereira, J. & Calabrese, A., 1983. Monitoring water quality at a dredge spoil dump site using oyster larvae. – C. M./ICES, *E 59,* 1–9.

Okubo, K. & Okubo, T., 1962. Study on the bioassay method for the evaluation of water pollution. II. Use of the fertilized eggs of sea urchins and bivalves. – Bull. Tokai reg. Fish. Res. Lab. *32,* 131–140.

Oshida, P. S., Bay, S. M., Haeckl, A., Goochey, T. K. & Greenstein, D., 1982. Sea water and waste water toxicity studies. – Coast. Wat. Res. Proj. biann. Rep. *1981–1982,* 217–223.

Pearson, T. H., 1975. The benthic ecology of Loch Linnhe and Loch Eil, a sea-loch system on the west coast of Scotland. 4. Changes in the benthic fauna attributable to organic enrichment. – J. exp. mar. Biol. Ecol. *20,* 1–41.

Rachor, E., 1977. Faunenverarmung in einem Schlickgebiet in der Nähe Helgolands. – Helgoländer wiss. Meeresunters. *30,* 63–651.

Sokal, R. R. & Rohlf, F. J., 1969. Biometry. Freeman, San Francisco, 776 pp.

Stebbing, A. R. D., Akesson, B., Calabrese, A., Gentile, J. M., Jensen, A. & Lloyd, R., 1980. The role of bioassays in marine pollution monitoring. – Rapp. P.-v. Réun. Cons. int. Explor. Mer *179,* 322–332.

Stebbing, A. R. D. & Brown, B. E., 1984. Marine ecotoxicological tests with coelenterates. In: Ecotoxicological testing for the marine Environment. Ed. by G. Persoone, E. Jaspers, & C. Claus. Lab. Biol. Res. Aquat. Pollut., State Univ. Ghent, Ghent, *1,* 307–339.

Thain, J. E. & Watts, J., 1984. The use of a bioassay to measure changes in water quality associated with a bloom of *Gyrodinium aureolum* (Hulbert). – C. M./ICES, *D3,* 1–14.

Waterman, A. J., 1937. Effects of salts of heavy metals on the development of the sea urchin, *Arbacia punctulata.* – Biol. Bull. mar. biol. Lab., Woods Hole *73,* 401–420.

Weichart, G., 1975. Untersuchungen über die Fe-Konzentration im Wasser der Deutschen Bucht im Zusammenhang mit dem Einbringen von Abwässern aus der Titandioxid-Produktion. – Dt. hydrogr. Z. *28,* 49–61.

Woelke, C. E., 1972. Development of a receiving water quality bioassay criterion based on the 48-hour Pacific oyster (*Crassostrea gigas*) embryo. – Tech. Rep. Wash. Dep. Fish. *9,* 1–93.