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Barentsia matsushimana, a Marine Entoproct Suitable for Bioassays

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Determination of the acute toxicity of environmental chemicals is only a first step towards the assessment of a possible threat to the environment. Responsible environmental management requires basic data about the biological behaviour. Longterm exposure studies at sublethal concentrations will minimize the risks connected to forecasting the future behaviour of substances in the environment. Biotests employing sensitive organisms or measuring sensitive criterions (McIntyre and Pearce 1980; Persoone et al. 1984; Scholz 1987b) are useful tools for preventive action. The following paper describes experiences gained during the development of standard test systems (Kayser 1982; Scholz 1986) with the marine entoproct Barentsia matsushimana. Though it may seem strange at first glance to employ a rare species like a marine kamptozoon, several features render B. matsushimana especially useful for this purpose:

- easy of rearing (Scholz,1987a)
- world wide geographical distribution (Toriumi, 1951; Emschermann, 1961)
- formation of resting buds
- possibility of genetic clones

As a preliminary step, reference chemicals, $K_2Cr_2O_7$ and 4 - nitrophenol were tested in order to check the sensitivity of the colony growth.

MATERIALS AND METHODS

For growth experiments resting buds were isolated from laboratory grown colonies of B. matsushimana. Up to 7 resting buds were attached to plastic slides (Trovidur) by means of a silikone paste droplet. The slides were transferred to petri dishes con -

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taining test chemicals in increasing concentrations. To induce germination, the buds needed a low temperature treatment at 5°C for 4 days. After this time they were brought to 15°C. Two weeks later, each resting bud had produced one to three calyces.

For further development and growth, the newly established colonies had to be fed. They were either transferred to a semistatic system in Erlenmeyer flasks or suspended in a flow-through system. In the Erlenmeyer flasks, water was changed twice a week and aliquots of a Phaeodactylum tricornutum stock culture were added to give a final concentration of $1.6 * 10^5$ cells ml^{-1}. A continuous algae culture of P. tricornutum ensured a final concentration of $1.2 * 10^5$ cells ml^{-1} in the flow-through test vessels.

The number of calyces was counted weekly. Total experimental time was 49 days (7 weeks), after which the number of newly established calyces per colony and per day was calculated for each experimental set. A total of 3 repetitions of 5 parallels for each concentration plus the additional controls were run. Test temperature and salinity were 15°C and 30‰ S respectively.

RESULTS AND DISCUSSION

In order to ascertain the ability of kamptozoans for use in standardized bioassays, growth experiments were conducted with Barentsia matsushimana. In the first steps, reference chemicals were employed. For practical reasons, experimental time was limited to 49 days. This time is long enough to cover a significant life span of the colony and short enough to hold secondary disturbances as low as possible. Examplary for an experiment with different Cr^{6+} concentrations, a typical growth pattern is demonstrated in Fig. 1. The linear increase in the number of calyces (logarithmic scale) is evidence for a steady colony growth, independent of chromium concentrations.At the end of the exposure time (49 days), the number of calyces established per colony and per day is calculated for each chromium concentration. The results are summarized in Table 1.

Growth was lower in both flow-through experiments. This was true in the controls and, although less pronounced, in the chromium spiked colonies. It may be caused by the colonies in the flow-through systems being overfed, exceeding the optimal algae concentration of about 100,000 cells of P. tricornutum ml^{-1} (Scholz 1987a). To eliminate the discrepancy the controls of each of the repetitions are set to 100 and all other values are expressed as the percent thereof (Table 2). The graphic summary is documented in Fig. 2.

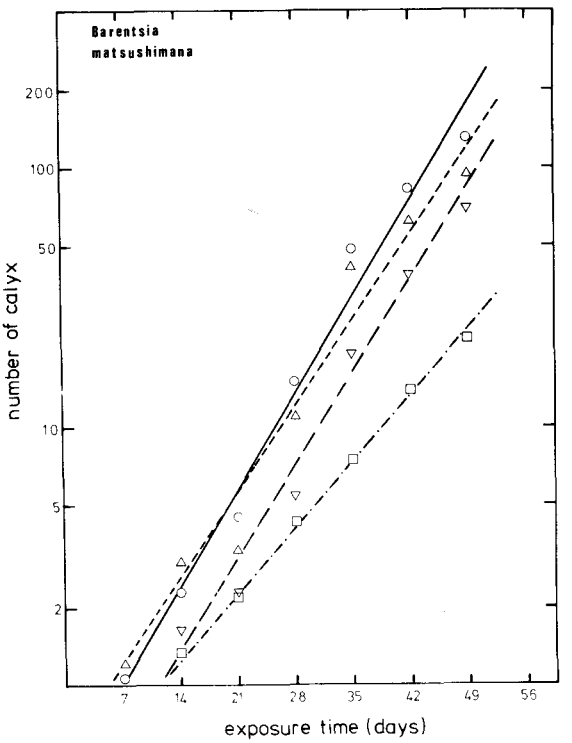

Figure 1. Barentsia matsushimana. Colony growth dependence on different Cr^{6+} concentrations; means and regression lines

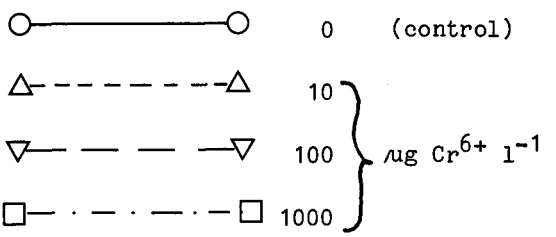

Table 1. Barentsia matsushimana. Number of calyces formed per day at different Cr^{6+}-concentrations. Mean +/- standard error. Experimental time: 49 days; n.d. = no data

Cr^{6+} (µg l^{-1})	semistatic (n = 6)	flow through (n = 5)	(n = 5)
0	2.61 +/- 0.33	1.46+/- 0.16	1.83+/- 0.17
10	1.82 +/- 0.36	n.d.	n.d.
100	1.42 +/- 0.34	0.90+/- 0.13	1.28+/- 0.16
1000	0.44 +/- 0.05	0.40+/- 0.04	0.42+/- 0.06

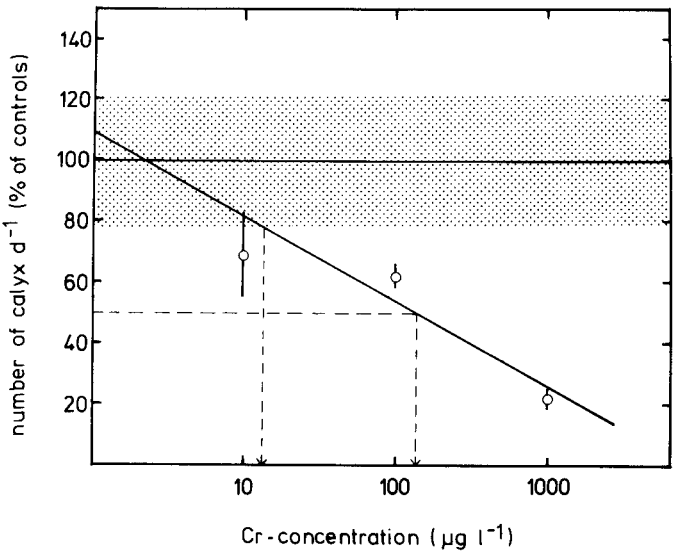

Figure 2. Barentsia matsushimana. Growth of colonies at different Cr^{6+}-concentrations, expressed as percent of the controls. Mean +/- standard deviation; shaded bar: controls with 95% confidence interval; experimental time: 49 days
Regression line: y = 109.4 - 27.7x; n = 7; r = -0.90922**

Table 2. Barentsia matsushimana. Influence of $K_2Cr_2O_7$ on the growth of the colonies, expressed as percent of their controls. Mean +/- standard error; n.d. = no data

Cr^{6+} (µg l^{-1})	semistatic (n = 6)	flow through (n = 5)	(n = 5)
0	100.0 +/- 12.6	100.0 +/- 11.1	100.0 +/- 11.0
10	69.7 +/- 13.8	n.d.	n.d.
100	54.4 +/- 13.0	61.8 +/- 14.0	69.7 +/- 12.4
1000	16.9 +/- 2.0	27.1 +/- 9.5	22.7 +/- 14.4

Following the same procedure, the results were calculated for the colonies grown under various 4-nitrophenol concentrations (see Fig. 3). No flow-through experiments were conducted in this case, however.

In a log - normal scale the relationship between concentration and growth retardation is linear (F-test for linearity, p=0.01). The upper and lower range of growth of the controls is confined to the 95% confidence interval, represented by the shaded bar in Fig. 2 and 3.

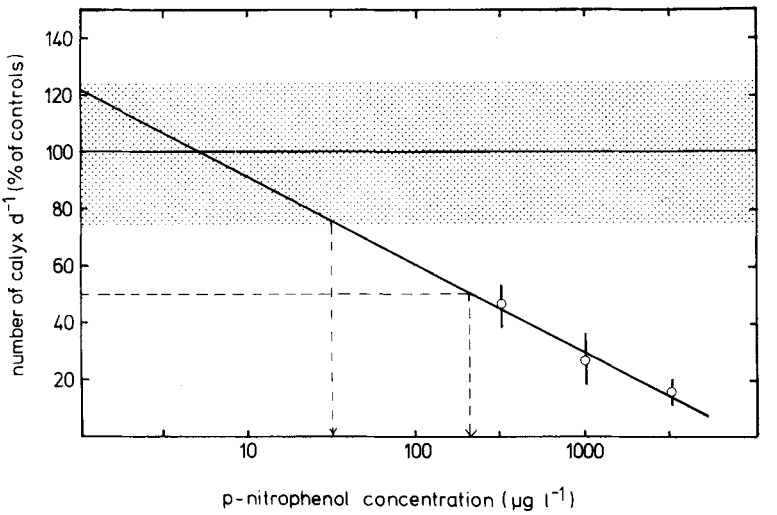

Figure 3. Barentsia matsushimana. Growth of colonies at different p-nitrophenol concentrations, expressed as percent of the controls. Mean +/- standard deviation; shaded bar: controls with 95% confidence interval; experimental time: 49 days
Regression line: $y = 122.1 - 31x$; $n = 12$; $r = -0.9942^{***}$

Significant deviations from control performances are documented by values lying outside of these confidence intervals. Stimulating effects would result in values above the upper edge, but were not observed here. Retarded development results in values below.

For quantitative comparisons, two criteria can be drawn from this relationship. One point of less importance is the EC_{50} value, which identifies the concentration responsible for a 50% retardation of growth. The biological and ecotoxicological significance of this concentration however is quite small. Of greater consequence is the intercept of the regression line with the lower edge of the confidence interval, which point represents the EC_m, the minimal effective concentration. Above this concentration, significant deviations from the control performance are to be expected. The EC_m values obtained for $K_2Cr_2O_7$ and 4-nitrophenol are summarized with the corresponding EC_{50} in Table 3.

Threshold values between different organisms should be compared with reservation only. Mostly, test criteria imply different biological manifestations at non-comparable levels, which in turn are of different significance for the species.

Table 3. Barentsia matsushimana. Minimal effective concentrations (EC_m) and EC_{50} of Cr^{6+} and 4-nitrophenol. Test criterion: colony growth

	EC_m	EC_{50}
Cr^{6+} ($\mu g\ l^{-1}$)	13.3	138.9
4-nitrophenol ($\mu g\ l^{-1}$)	33.3	211.7

Keeping this in mind, the sensitivity of B. matsushiamna seems to be exceptionally high in the case of 4-nitrophenol, lying one order of magnitude below the NOEC values obtained in an intercalibration test with Daphnia magna (Anon 1985). In the case of $K_2Cr_2O_7$, B. matsushimana displays a sensitivity comparable to those found in other bioassays. NOEC values between 10 and 100 $\mu g\ l^{-1}$ were found in D. magna (Anon 1985). And values between 40 and 100 $\mu g\ l^{-1}$ have been proved to surpress the reproduction of marine polychaetes (Oshida and Word, 1982).

Considering these first results, some necessary features for a biotest system are combined in Barentsia matsushimana. Its easy, year-round availability as in Artemia salina combined with a high sensitivity as in hydrozoans (Theede et al. 1979) proves this species suitable for bioassays with marine organisms. More intensive work has to be done in order to optimize test conditions and to obtain more insight into the general biology and autecology of Barentsia matsushimana.

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