Bull. Environ. Contam. Toxicol. (1985) 35:660–665

Bulletin of Environmental Contamination and Toxicology

Induction of Chromosomal Aberrations in the Mussel *Mytilus galloprovincialis* Watch

Kabil Al-Sabti and Branko Kurelec*

Center for Marine Research Zagreb, Rudjer Bošković Institute, POB 1016, 41001 Zagreb, Croatia, Yugoslavia

Filter and absorber-feeding, a sessile way of life in a spectrum of environmental conditions, world wide distribution, availability, and ease in laboratory keeping, make the mussel Mytilus galloprovincialis an ideal organism in pollution oriented research. Mytilus accumulates genotoxic hydrocarbons which may be extracted and quantified by chemical or biological methods (Parry et al. 1976, Parry and Al-Mossawi 1979). Mussels can activate premutagens, as was found by chemical analysis, (Stegeman 1981) as well as by mutagenicity testing of the metabolic products (Anderson and Döös 1983). This potential has been exploited in a mussel--host mediated assay aimed at the detection of premutagens in sea water (Frezza et al. 1982). Bioactivation of carcinogens to their ultimate forms is responsible for at least a part of neoplasms found in mussels (Alderman et al. 1977, Harshbarger 1977, Mix et al. 1979), as well as for the induction of chromosomal structural aberrations and sister chromatid exchanges (SCE) (Dixon and Clarke 1982).

In this paper we present an investigation into the occurrence of chromosomal aberration (CA) induction in mussels. The feasibility of using this as an indicator of genotoxins under actual field conditions has been evaluated.

MATERIAL AND METHODS

Specimens of the adult common mussel (Mytilus galloprovincialis) 5 to 7 cm in shell length, were collected from the low water neap tide level in the area of Rovinj, in the Northern Adriatic, at several sites that are affected by local sources of mixed organic pollution (Fig. 1). Within one hour they were used for chromosomal preparations. The specimens collected in the Lim channel served as controls and as material for exposition at other sites ("mussel watch" - type experiment, Goldberg et al. 1978). Ten specimens, held in a nylon net, anchored at the low neap tide level at selected sites were exposed for two days. Groups of 10 specimens were used in laboratory exposures to sea water polluted with benzo(a)-pyrene (BaP) for 2 days in 600 ml glass beakers. Different concentrations of BaP (purchased from Roth, Karlsruhe, FRG) were prepared by dissolving

* Correspondence and reprint author

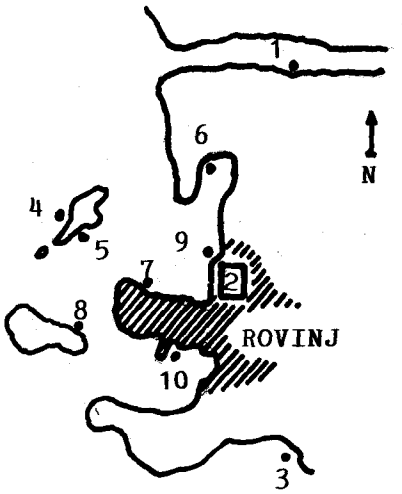

Fig.1. Collection and exposure sites

BaP in acetone as a stock solution. From this, the desired amount was applied to the sea water at time zero and after 24 hours.

Preparation of chromosomes was according to the method of Dixon and Clarke (1982) with slight modifications as described by Al--Sabti et al. (1983). The freshly collected or exposed mussels were exposed to 0.04% colchicine in clean sea water for 6 hours, the gills excised and treated with 50 and 25% sea water subsequently for 30 min each. The tissues were fixed in Carnoy (3 ethanol : 1 glacial acetic acid), placed onto a clean and cold (-5°C) slide, homogenized by forceps until tissue turned into a homogeneous emulsion, dried on a flame, left at room temperature for 3 h and stained with 20% Giemsa solution for 7 min. The gills of each specimen were prepared on a separate slide. Such slides were examined under the microscope (OPTON, FRG) and photograph taken at a magnification of 10X and 100X on an EFKA 14 (135--36) film. Seven to nine mussels were analysed per group. On the average, 20 metaphases have been detected per 3.200 microscopial fields scored on each slide. Slides were scored "blind" in order to avoid bias. One slide took 2 h scorer time, or, 2 scorer days per group. Theoretically, the results presented in Table 1-3 require 44 full-time days for scoring, or more than two scorer months.

In this work we counted breaks and fragments only, since ring and dicentric chromosomes represented less than 3% of the aberrations. Regardless of whether there was a mono- or poly-aberration in one mitosis, it was evaluated as one single aberrant mitotic figure.

RESULTS AND DISCUSSION

The frequency of aberrant mitophase cells in the gills of M. galloprovincialis from different sites in the Rovinj area are given in Table 1, together with its statistical evaluation. The maximal frequency of CA found in a natural population was 7.7 ± 2.7 (site 9). Since the number of aberrations found in the specimens from the Lim channel was the lowest, their frequency of CA of 2.9 ± 2.8% was used as the control rate of CA - appearance.

In the "mussel watch" experiment, the M. galloprovincialis transferred for 2 days to polluted sites induced an increased number of CA. The highest induction of CA was found in mussels transferred to the harbour of Rovinj, site 10 (3.5 fold) and the Institute pier, site 9 (2.8 fold). Neither site is inhabited by mussels. The induction rate in mussels transferred to other sites closely ap-

Table 1. Frequency of chromosomal aberrations found in the populations of Mytilus galloprovincialis from different localities in the Rovinj area

Site*	No. of specimens analyzed	No. of counted metaphases	% of aberrant metaphases
1	9	448	2.9 ± 2.8
2	8	221	4.1 ± 2.3
3	9	192	6.3 ± 2.0
4	7	227	4.4 ± 2.9
5	7	242	4.1 ± 3.5
6	8	230	6.1 ± 4.1
7	7	195	7.7 ± 2.7
8	8	189	5.3 ± 3.3

*Single-tailed t-test on the relative differences of means in aberrant metaphases yields, testing 1 : 8, $p \succ 0.2$; 1 : 2, $p \succ 0.005$; 1 : 3, $p \succ 0.025$; 1 : 9, $p \succ 0.005$; 8 : 2, $p \succ 0.05$; 8 : 3, $p \succ 0.2$; 8 : 9, $p \succ 0.01$.

Table 2. Induction of chromosomal aberrations in the naive Mytilus galloprovincialis exposed for two days to waters at different sites in the Rovinj area

Site	No. of specimens analyzed	No. of counted metaphases	% of aberrant metaphases
1 (control)	8	243	2.9 ± 2.8
1 (exposed in net)	9	232	3.9 ± 1.9
2	9	235	3.8 ± 2.3
3	8	224	5.4 ± 3.7
4	7	213	3.8 ± 1.9
5	7	223	3.1 ± 1.9
6	9	203	5.9 ± 3.8
7	8	191	6.8 ± 3.9
8	9	211	4.2 ± 3.1
9	7	199	8.0 ± 4.5
10	7	169	10.1 ± 6.2

proaches that of the resident population (Table 2). Note that the maximum number of aberrant metaphase cells observed in any of the mussels transferred to sites inhabited with mussels was 6.8 ± 3.9% (site 8).

In the groups of mussels exposed for 2 days to 1, 5 and 10 ppb of BaP, a dose-response of CA induction was observed (Table 3).

The area under investigation in this work is quite well defined with regard to its pollutional loads. The harbour of Rovinj (site

Table 3. The effect of exposure of Mytilus galloprovincialis from the Lim channel to different benzo(a)pyrene (BaP) concentrations for 48 h

Exposed to	No. of specimens analyzed	No. of counted metaphases	% of aberrant metaphases*
Institute pond	8	231	3.0 ± 2.8
1 ppb BaP	6	217	8.8 ± 6.8
5 ppb BaP	6	209	20.6 ± 15.4
10 ppb BaP	6	181	48.6 ± 30.5

*The correlation coefficient was $p = 0.392$, yielding significance to the existing correlation between the concentration of BaP added, and the percentage of aberrant metaphases at $p < 0.025$.

10, Fig. 1) is a recipient of untreated domestic and typical harbour wastes. At site 9 there is a fluctuating source of pollution delivering untreated wastes from a fish cannery. The influence of these source is visibly recognizible at sites 4, 5 and 6. During the short summer tourist season an incrase in the discharge of wastes also affects site 3. The Lim channel (site 1) as a protected area is considered to be "clean", as well as Institute pond water (site 2).

The numbers of CA in the gills of mussels collected in August 1983 at these points reflect in an expected way the quality of their respective environments (Table 1). Thus for at least sites 5, 6 and 9 the numbers of CA found are consistent with the levels of benzo(a)pyrene monooxygenase activity determined in the livers of the local fish Blennius pavo (Kurelec et al. 1977) and with the levels of hexane extractable xenobiotica and mutagenic substances, as demonstrated in the BPMO-induct test and in the Ames-microsomal test (site 9, Kurelec et al. 1979). According to these criteria the quality of water in these areas decreases, in the following order of sites: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. The number of CA is at least doubled as compared to the basal rate in the mussels from the Lim channel when they are exposed at polluted sites.

The purpose of this work was to explore whether the principle of the "mussel watch" could be applied in genotoxicity assessment in areas which are not inhabited by mussels. Since the exposure of control mussels to benzo(a)pyrene increases the rate of CA induction in a dose-response manner within two days (Table 3), this period was chosen as the standard in the "mussel watch" experiment. Exposure of mussels collected in the Lim channel to waters at several points induce CA frequencies similar to the ones in the respective natural populations. Thus, the clastogenic effect of waters at sites not naturally inhabited by mussel such as the harbour and the pier at the Institute could be assessed by exposing there control mussels for two days (Table 2). Actually, at these sites the CA frequencies were the highest found, which is in agreement with

our knowledge about pollutional loads and toxic effects in this area.

These results indicate that the frequency of CA in the gills of mussels transferred to the new environment can serve as a relevant parameter in the assessment of genotoxic chemicals present. This relevance of CA even introduces a new quality: it indicates a consequence of DNA damage, mistakes in DNA repair or misreplication (Evans 1977, Obe et al. 1982). It has the advantage over SCE in that it is applicable under field conditions. In addition it does not suffer from the inability of SCE to detect certain chemicals (Thilagar and Kumaroo 1983, Ivett and Tice 1981, Anderson et al. 1981).

Acknowledgements. Financial support of the Self-Managing Community for the Scientific Research of Croatia in gratefully acknowledged.

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Received December 26, 1984; accepted January 7, 1985