WATER QUALITY AND STRESS INDICATORS IN MARINE AND FRESHWATER ECOSYSTEMS: LINKING LEVELS OF ORGANISATION (INDIVIDUALS, POPULATIONS, COMMUNITIES)

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Mussel eggs as indicators of mutagen exposure in coastal and estuarine marine environments

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The aim of this study was to develop a short-term genotoxicity assay for monitoring the marine environment for mutagens. Based on the developing eggs and embryos of the marine mussel *Mytilus edulis*, an important pollution indicator species, the test employs the sensitive sister chromatid exchange (SCE) technique as its end-point, and exploits the potential of mussel eggs to accumulate mutagenic pollutants from the surrounding sea water. Mussel eggs take up to 6 months to develop while in the gonad, which provides scope for DNA damage to be accumulated over an extended time interval; chromosome damage is subsequently visualised as SCEs in 2-cell-stage embryos after these have been spawned in the laboratory. Methods which measure biological responses to pollutant exposure are able to integrate all the factors (internal and external) which contribute to the exposure. The new cytogenetic assay allows the effects of adult exposure to be interpreted in cells destined to become part of the next generation.

Introduction

Each year more than 500 newly-synthesised chemicals are released into the environment (Bridges 1971; Alink 1982). Many of these chemicals ultimately find their way into the aquatic environment (i.e. rivers, seas and oceans) through a variety of routes: waste disposal and effluents, surface water run-off, and from gaseous and particulate discharges. A significant proportion of these chemicals have the potential to damage DNA, either directly or through disturbance of the processes involved in DNA synthesis and repair (Sorsa & Vainio 1982).

The biological consequences of DNA damage are threefold: (1) it may result in cell death, (2) DNA repair processes may restore the damaged DNA to its original structure, (3) in the course of repair or replication a permanent alteration to the DNA molecule may arise (i.e. a mutation). If one accepts the evidence that mutations are the primary cause of neoplasia (Magee 1977; Mix 1986), then short-term tests designed to detect mutagenic activity can be valuable tools to screen for carcinogenic substances in the aquatic environment. While SCEs (sister chromatid exchanges) are not mutational events in the conventional sense, since the chromosome involved remains morphologically unchanged, they have been shown, both *in vitro* and *in vivo*, to be extremely sensitive indicators of chromosomal disturbance, often at mutagen concentrations several orders of magnitude below those at which significant increases in structural and numerical aberrations become apparent (e.g. Pesch *et al.* 1981; Dixon & Clarke 1982). It is the subtle, non-lethal changes in chromosome structure which represent the real risk to the next generation through the production of genetically defective gametes and embryos. An extensive literature, based on a variety of *in vivo* and *in vitro* test systems, supports the use of SCE as a sensitive genetic end-point for use in environmental monitoring (see collected papers in Tice & Hollaender 1984a,b).

Genotoxic agents occur in the aquatic environment most commonly at extremely low concentrations (Parry *et al.* 1976; Galassi *et al.* 1992). Except for pollution hot-spots, these concentrations lie almost without exception below the lower limit of detection of the

cytogenetic methods currently available. Consequently, there is a need to either develop more sensitive methods or utilise aspects of the biological material to increase sensitivity, for example by integrating the effects of exposure over an extended time period (Parry *et al.* 1980; De Flora *et al.* 1991).

The approach we have opted for utilises the developing egg (i.e. oocyte) of the marine mussel, *Mytilus edulis*, which takes several months to develop while in the gonad and has the potential to accumulate a range of mutagenic pollutants (Parry *et al.* 1976; Pipe 1987). The mussel occupies a prominent position in marine pollution monitoring studies (e.g. Goldberg 1975; Bayne 1976; Bayne *et al.* 1985). Its cosmopolitan distribution, sedentary habit (as an adult), ecological abundance, filter-feeding behaviour, relatively large size, and ability to accumulate heavy metals, polycyclic and halogenated aromatic hydrocarbons, and radionuclides, make mussels ideal candidates for water-quality assessment studies (Roberts 1976; Phillips 1980; Krieger *et al.* 1981; Valerio & D'Ambrosio 1989).

Since the first use of the mussel in chemical monitoring (Goldberg 1975), considerable scientific effort has been expended in developing a range of complementary, sub-lethal test protocols, using it in the capacity of an aquatic "white rat" (e.g. Bayne *et al.* 1985). This approach includes the full range of biological effects, from whole organism responses (e.g. Widdows & Donkin 1992) to changes at the cellular and sub-cellular levels (e.g. Moore 1990; Livingstone *et al.* 1991), including changes to the DNA, the genetic material. Interference with the genetic blueprint is potentially the most serious aspect of marine pollution since it threatens not only the health of present-day populations but also has the potential to undermine the fitness of future generations (Beardmore *et al.* 1980; Dixon 1985; World Health Organization, United Nations Environment Programme 1989).

Materials and Methods

The karyotype

Mussels belonging to the *Mytilus edulis* species complex, which includes *M. galloprovincialis* Lmk., have a diploid karyotype consisting of 28 chromosomes, size range 3–7 μm (Moynihan & Mahon 1983; Thiriot-Quiévreux 1984; Dixon & Flavell 1986; Pasantes *et al.* 1990). Mussel chromosomes are sufficiently large and the karyotype sufficiently heteromorphic to allow detailed microscopical analysis (Dixon & Clarke 1982; Dixon & Prosser 1986; Dixon *et al.* 1986; Pasantes *et al.* 1990).

Sister chromatid exchange (SCE) methods

(a) Maintenance and spawning of adult mussels. Adult animals used in this study were normally held in clean, aerated sea water and fed a daily ration of the algae *Phaeodactylum* and *Tetraselmis*. All pre-spawning manipulations were carried out at 8–10°C to inhibit the mussels from spawning prematurely. Ripe mussels were induced to spawn by temperature shock, i.e. immersion in sea water at 26°C for 20 minutes (Harrison & Jones 1982), after which they were placed in individual beakers to release their gametes. Males, which generally commenced releasing gametes in advance of females, were wrapped in damp tissue and held in a refrigerator until their sperms were needed for fertilizations. All laboratory experiments were carried out at 15°C; at this temperature a maximum frequency of 2–cell-stage embryos was usually achieved after approximately 4 hours.

(b) BrdU treatment. Initially, to achieve differential labelling of the sister chromatids, adult mussels were exposed for 5 days to bromodeoxyuridine (BrdU; Sigma) at a concentration of 20 mg l^{-1}, followed by two further exposures of the spawned egg culture (100 eggs ml^{-1}) to BrdU,

each at 100 mg l^{-1}, for approximately 3 hours duration, pre- and post-fertilization. However, by introducing a 5 N HCl step into the staining protocol (Kerkhoff & Gaag 1985), it was eventually found possible to reduce the BrdU treatment to simply the post-spawning exposures, the second BrdU treatment being delayed until 60 min after the first introduction of the sperms, to reduce any interference with the fertilization process (Dixon 1983a).

Figure 1 shows how the semi-conservative replication of DNA in the presence of 5-bromodeoxyuridine (BrdU), a DNA-base (thymine) analogue, underlies the differential labelling of sister chromatids (Latt 1974; Perry & Wolff 1974) and how this procedure was incorporated into the first two cell divisions of the developing mussel embryo. Chromatids (chromosome arms) which have both strands of their DNA molecule substituted with BrdU have increased sensitivity to UV-light and are pale in appearance when Giemsa-stained following this treatment. Under normal conditions this differential staining pattern between partially and fully substituted chromatids, in chromosomes which have undergone two divisions in the presence of BrdU, is rigorously conserved. In contrast, chromatids in chromosomes which have been exposed to mutagenic chemicals, UV-light or irradiation, display a characteristic "harlequin" pattern of staining, caused by the reciprocal exchange of chromosome arm material (Kato 1974; Latt 1974; Perry & Evans 1975). It is the number of these so-called sister chromatid exchange events (SCEs) which can be used as a sensitive indicator of mutagen exposure (Fig. 2).

(c) Colchicine and hypotonic treatments. Embryos destined for chromosome analysis were transferred to a nitex filter (20 μm mesh) and immersed in a 0.1% colchicine solution in filtered sea water for 60 min. This was followed by a series of hypotonic treatments based on dilutions of 0.075 M KCl and filtered sea water in the ratios 1:2, 1:1, 2:1 and 4:1, 10 min each. Towards the end of the last hypotonic treatment, the embryos were concentrated into a loose pellet by slow centrifugation, 180 g for 2–3 min.

(d) Trypsin treatment and fixation. To improve the spreading of embryos during slide making, these being surrounded by a tough cuticle (Dixon 1983a), they were were first incubated at room temperature in 2 ml of the 4:1 hypotonic solution to which was added 2 ml of Bacto-trypsin solution, made up in the same hypotonic solution (i.e. 10 ml per vial). After 6 min exposure to trypsin, the softened embryos were recovered by slow centrifugation, 180 g for 2–3 min, and fixed. Fixation was carried out, after all the supernatant sea water had been carefully removed, in fresh, cold (i.e. 4°C) Carnoy's fixative (methanol: glacial acetic acid, 3:1), with three changes over an hour. Slides were made shortly after fixation, which prevented the embryos from hardening in the fixative.

(e) Slide making. A few drops of concentrated embryo suspension in Carnoy's fixative were transferred to a clean watch-glass containing 5 volumes of 60% acetic acid. The embryos were left to "clear" in the dilute acid for several minutes before transferring to a clean microscope slide on a hotplate, at 40°C. Excess fixative was allowed to evaporate off before a second glass slide was placed over the embryos, which were squashed by rotating the slides in opposite directions. After separation the slides were replaced on the hotplate, which caused the drops of liquid to round off in the heat. The liquid drops were dislodged by briskly striking the slides sideways-on against the bench. Number and quality of the chromosomes were initially checked using a medium-power, phase-contrast objective.

(f) Slide staining. Throughout these experiments it was our practice to stain slides in Hoechst 33258 (45 μg per ml) in PBS buffer (8 g NaCl, 0.2 g KCl, 0.2 g KH_2PO_4, 1.4 g Na_2PO_4 per litre) for 10 min, followed by a further 60 min in fresh Hoechst solution under a UV-lamp (see

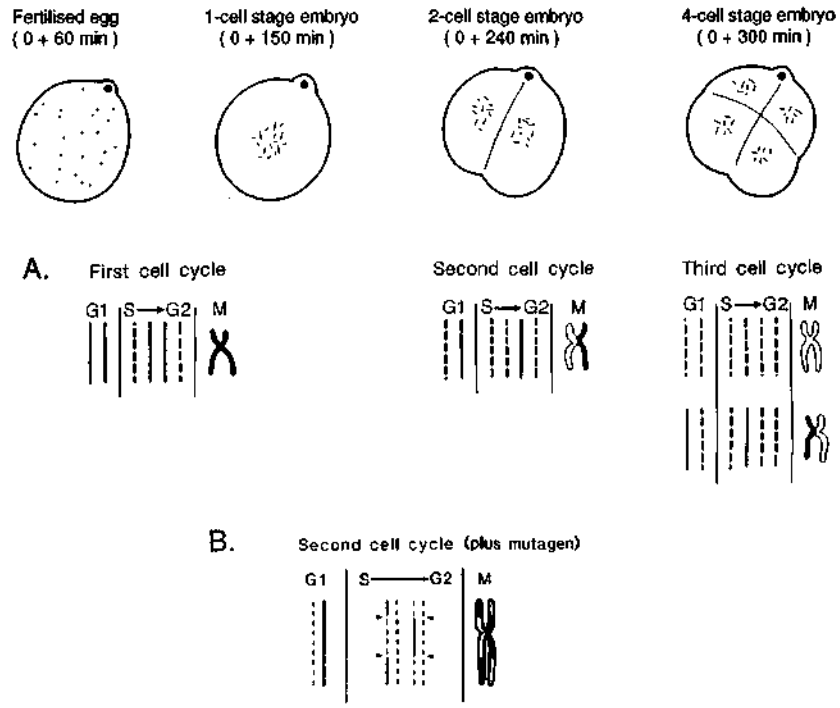

Figure 1. Development in embryos of mussels (*Mytilis edulis*) up to the 4–cell stage, showing: A, the accompanying pattern of BrdU incorporation (dashed lines) responsible for sister chromatid differentiation, and B, the effect of a mutagen, inducing SCEs in the second cell cycle. Phases in the cell cycle are represented by: G1 = gap 1; S = synthesis of DNA; G2 = gap 2; M = mitosis. Chromosomal regions exhibiting Giemsa-staining contain DNA which is only partially substituted with BrdU; pale-staining chromosome arms represent DNA which is completely substituted, i.e. incorporated into both polynucleotide chains.

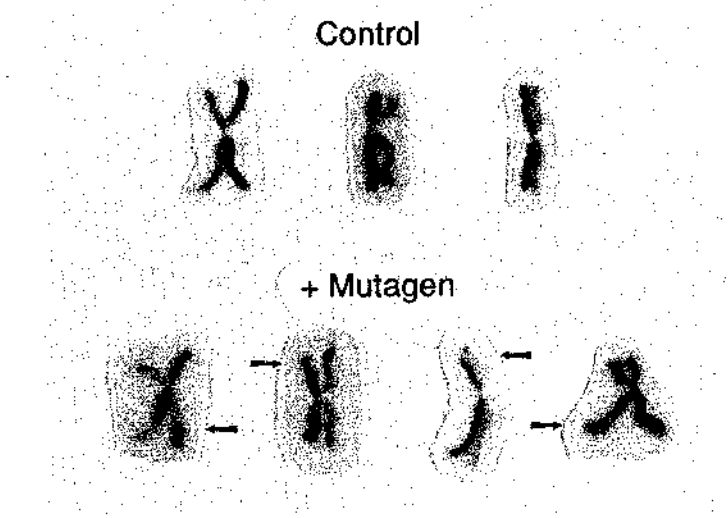

Figure 2. Individual chromosomes from embryos of *Mytilis edulis* at the 2–cell stage, showing sister chromatid exchange events (SCEs; arrowed).

Dixon & Clarke 1982). More recently we have dispensed entirely with the use of the Hoechst stain since this fluorochrome is not required for SCE work if the slides are going to be examined under bright field after Giemsa staining. To remove surface protein contamination, which will otherwise obscure the differential staining, the slides were incubated in 5 N HCl for 25 min (Kerkhoff & Gaag 1985). After the acid treatment, the slides were rinsed with distilled water before staining for 6 min in 10% Giemsa stain (Gurr's Improved R66), in pH 6.8 buffer, followed by blueing in tap water which contained a few drops of ammonium hydroxide solution.

(g) Microscopic analysis. This was carried out using x63 and x100 oil-immersion lenses and a green filter to enhance contrast. To improve resolution, coverslips and mounting medium were dispensed with in this study. With the improvements in technique (see above) the entire process, from spawning to slide making, took only about 8 h per sample. With replicated glassware, four pair-crosses could be achieved comfortably per day. Approximately one thousand chromosomes were scored per treatment; 2–cell-stage embryos did not yield intact metaphases.

Laboratory studies

Two initial studies of relevance to the development of the SCE method were devised; first, to analyse the effect of BrdU treatment on the cell kinetics of early embryos, and second, to compare the SCE levels in 2–cell-stage embryos, after exposure of adults or spawned eggs and embryos to a standard mutagen.

(a) Effects of BrdU on development rate. Mussels collected from a genotoxically clean site (Whitsand Bay, S.E. Cornwall; see Dixon 1982) were spawned and egg cultures, representing five separate pair-crossings, were treated with BrdU at 100 mg l^{-1}, both before (3 h) and after fertilization. A replicate control series was used without the addition of BrdU. Aliquots of eggs/embryos were removed at intervals and scored for development stage, 100 individuals being scored for each sample.

(b) Exposure of adults and spawned eggs to a standard mutagen. Two separate experiments were carried out using mitomycin C (MMC), a commonly-used, standard mutagen which does not require metabolic activation (Latt 1974).

For adults, groups of 10 reproductively mature mussels (3–4 cm shell length) were kept for 5 days in 5-litre volumes of millipore-filtered (0.4 μm), aerated sea water containing MMC at different concentrations. Each group was fed a daily ration of *Phaeodactylum* and *Tetraselmis*, sufficient to maintain their reproductive condition (Dixon & Prosser 1986). Two groups were exposed to MMC at concentrations of 6×10^{-8} and 6×10^{-7} M respectively. A third group acted as a negative control and received only clean sea water. The water in the tanks was changed daily, at which time fresh MMC was added. After the 5-day exposure, adults were spawned and the eggs and embryos treated according to the SCE methods above.

Experiments on eggs and embryos were carried out in covered beakers containing a 1-litre volume of an egg suspension, at a concentration of approximately 100 eggs ml^{-1}, in aerated sea water. Three treatments were used: Mitomycin C (MMC), at two concentrations, 6×10^{-8} and 6×10^{-6} M, for 3 h pre- and post-fertilization, and a control without MMC. The egg suspension was fertilized using approximately 2 ml of concentrated sperm suspension. BrdU was used in conjunction with the MMC, and SCE preparations were produced and scored as described above.

Field studies

Field sampling of native mussel populations was carried out in the spring of 1987 (Fig. 3). Two reference sites were selected as being "genotoxically clean": Whitsand Bay, S.E. Cornwall, which has featured in previous studies (Dixon 1982; Dixon & Prosser 1986), and Brancaster Harbour near The Wash, Norfolk, which has been identified as a source of clean animals for scope-for-growth transplant studies conducted at this laboratory (J. Widdows, *pers. comm.*). The River Fal (3 stations), S. Cornwall, a well documented heavy-metal polluted estuary (Bryan & Gibbs 1983), and the mouth of the Red River, in St Ives Bay, N,W. Cornwall, a region heavily contaminated with mining waste, were selected as comparatively polluted sites. (Note: large-scale mining waste inputs into the Red River ceased in 1988; G.W. Bryan, *pers. comm.* 1993). All the mussels were transported to the laboratory on ice. During a short laboratory holding period, not exceeding 18 h, the mussels were kept dry in a coldroom at 5°C. This treatment prevented them from spawning and also prevented any loss of pollutants through depuration. Great care was taken to limit the length of this holding period and to avoid exposure to high temperatures, both of which are known to dramatically increase the levels of genotoxic damage in this organism (Dixon unpublished; see also Brunetti *et al.* 1992). Ripe adults from all sites were induced to spawn and values for SCE levels in their embryos were obtained as described above. Unfortunately, the mussel populations in the Fal and Red rivers were extremely sparse and the frequency of sexually mature individuals was generally very low. Consequently, only a few ripe specimens were found for spawning purposes. Bryan *et al.* (1985) have already drawn attention to *Mytilus* being rare in many UK estuaries which tend to be dominated by sediments, thus representing a major disadvantage for their use as indicators.

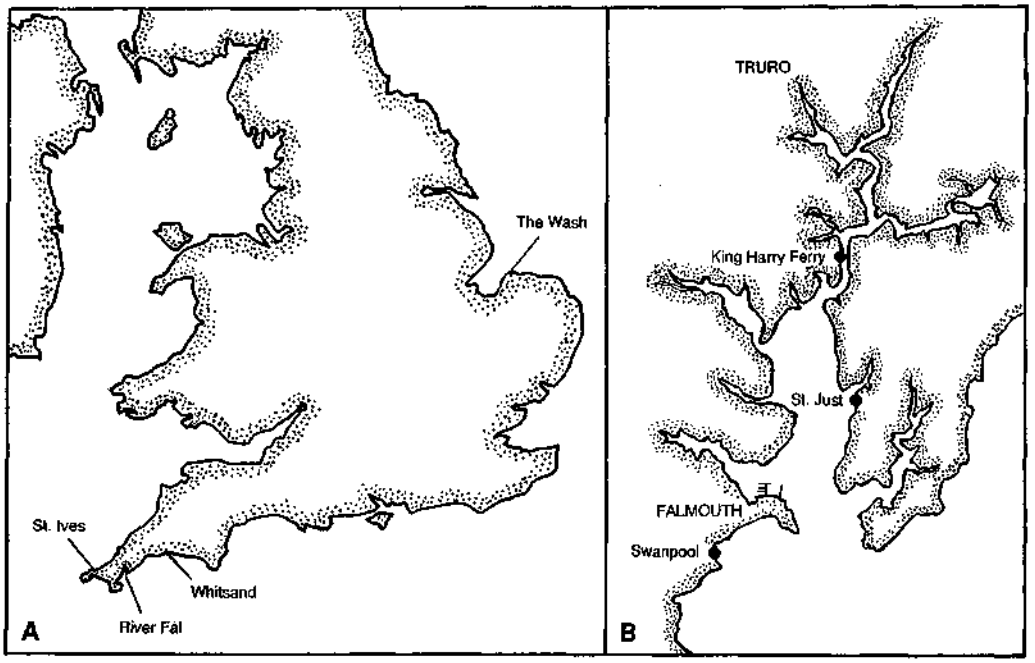

Figure 3. A, Field sites featured in the present study; B, three sites (●) within the River Fal (King Harry Ferry, St Just and Swanpool).

Results

Cell kinetics

Figure 4 shows the effects of 100 mg l^{-1} BrdU on mussel embryo development rate, at 15°C. While there was variation in both the control and treatment groups with respect to the rates of development of the individual pair-crosses, there was evidence of a reduction in development rate in those embryos exposed to BrdU. A Mann-Whitney U-test was carried out on the time at which the 2-cell embryo stage peaked (p = 0.08); hence there is mild evidence (significant difference at the 10% level) for a difference between the BrdU and control treatments. Based on these findings, a post-fertilization harvest time of 240 min was selected to maximise on the frequency of 2-cell stages. Mitotic delay is a common feature of toxicant exposure (Dixon 1985).

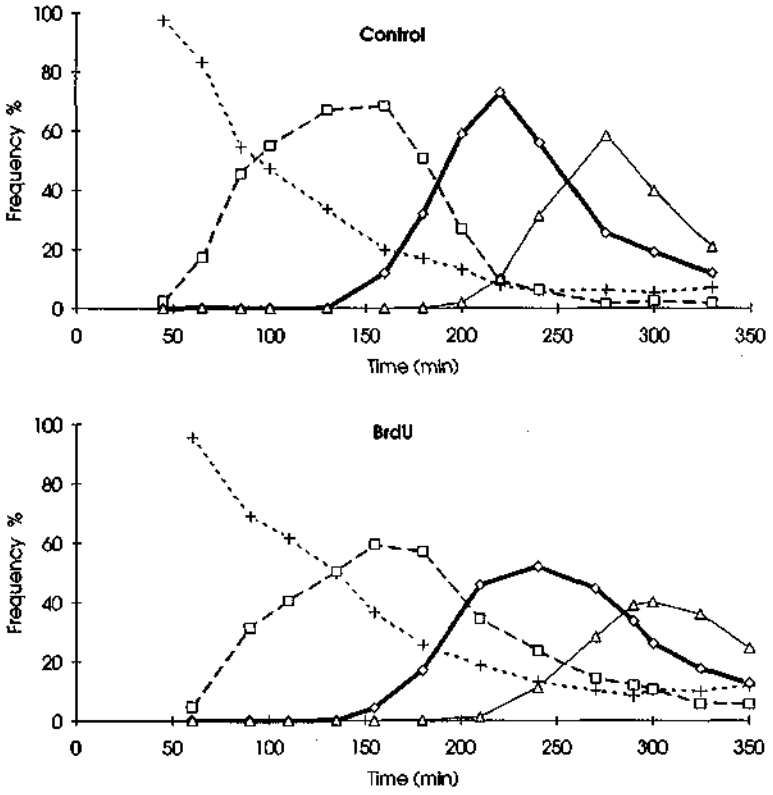

Figure 4. The effect of BrdU (100 mg l^{-1}) on early embryonic development of *Mytilus edulis*. Each point represents the mean of five separate pair-crosses. Symbols: +, egg not fertilized; □, polar body formed; ◇, 2–cell stage; △, 4–cell stage.

Effects of a standard mutagen

Figure 5 summarizes the results of the two experiments, with adults and spawned eggs exposed to MMC. The mean values plotted on Figure 5 refer to the averaged SCE results for five or six separate pair-crosses. It is clear that with increasing MMC dose there is a corresponding increase in SCE frequency in both exposed adults and acutely-exposed embryos. For the lowest

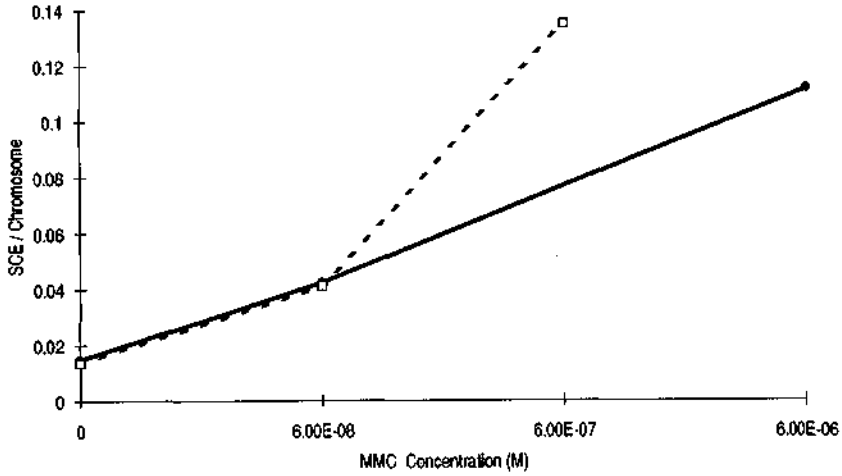

Figure 5. Effects of exposure to a standard mutagen (MMC = mitomycin C) on the frequency of sister chromatid exchange events (SCEs) in *Mytilis edulis* embryos at the 2–cell stage of development. The solid line (●) represents exposure of post-spawned eggs and embryos to MMC at molar concentrations of 6×10^{-8}, 6×10^{-7} and 6×10^{-6}. The broken line (□) represents the exposure of egg-carrying adult mussels to MMC.

MMC dose, there was no difference in the level of SCE recorded in mussel embryos exposed before or after spawning. [Note: 6×10^{-8} M MMC was found to induce a significant increase in SCE in adult mussel gill after 5 days exposure (Dixon & Clarke 1982) but did not cause a significant response in SCE frequency in larvae after only 12 h exposure (Harrison & Jones 1982)]. There was a significant increase in SCE frequency in embryos that were exposed, pre-spawning (i.e. as eggs in adults), to an intermediate dose of 6×10^{-7} M, compared with those which were exposed, post-spawning, to the higher dose, 6×10^{-6} M MMC (Fig. 5). To compare treatments a one-way ANOVA was carried out on the square-root transformed data; this revealed significant differences at the 1% level. These findings suggest that the extended exposure time which can be achieved using adult mussels confers greater sensitivity to the test method, and support the use of this approach in mutagenicity screening. The results are too preliminary, however, to cast any light on the question of the possible role of the mussel as an integrator of pollution (in this case, genotoxin) exposure (see Coleman *et al.* 1986). The low baseline SCE frequency in mussel embryos, compared with the values for the later life-history stages (Dixon & Clarke 1982; Harrison & Jones 1982), was similar to the relationship recorded for the serpulid tubeworm *Pomatoceros lamarckii* (Dixon & Pascoe, unpublished; see later).

Field studies

Table 1 shows the SCE results for the four polluted locations, and those for the two reference (i.e. genotoxically clean) populations. Irrespective of the preliminary nature of these data, it is apparent that there is a marked difference between the SCE levels recorded for the different field sites and the two reference populations. A one-way ANOVA was carried out on the square-root transformed field data; this revealed significant differences between the two polluted sites, and between the polluted sites and the two reference sites, at the 1% level. The highest SCE value was recorded for the Red River, the most chemically-polluted site at the time, where the major (recorded) contaminants are known to be copper and zinc; in contrast, all

three River Fal populations had similar, intermediate SCE values. There was a correlation between the SCE levels and the levels of heavy-metal pollution at the different sites (Table 1).

Table 1. *Relationships between sister chromatid exchange (SCE) frequency in 2–cell-stage embryos of* Mytilus edulis, *and tissue concentrations of copper and zinc in adult mussels from two reference sites and four contaminated sites (see the text).*

Values for Cu and Zn are given as μg per g dry wt.

Sites	2–cell embryos			Adults	
	SCE/chromosome	S.D.	No. of matings	Cu	Zn
Reference					
The Wash	0.022	0.0022	5	6.4	100.0
Whitsand	0.021	0.0073	5	9.4	153.0
Contaminated					
Swanpool	0.064	—	1	—	—
King Harry	0.068	—	1	—	—
St Just	0.069	—	1	—	—
Red River	0.204	0.127	2	212.8	508.8

Discussion

The marine environment receives a wide variety of contaminant inputs, including many chemicals and radionuclides which are either known or suspected to be mutagenic and/or carcinogenic. These agents present a risk of genetic damage to exposed species and those, including man, which are trophically dependant on the marine environment for food (World Health Organization, United Nations Environment Programme 1989). The level of risk posed by genotoxic pollutants to marine life is not altogether clear, although there is substantial evidence from a variety of different sources (e.g. Mix 1986; Bolognesi *et al.* 1992) which indicates cause for concern, and supports the need for the development of suitable test assays to detect and monitor the levels of this specific class of pollutants in marine habitats.

The developing egg (primary oocyte) of the mussel *Mytilus edulis* has a unique characteristic which confers this cell type with the potential for monitoring mutagens in the marine environment. As a result of the phenomenon of meiotic arrest (Longo & Anderson 1969), oocytes remain fixed at an early stage in cell division during their development which can last several months (Lowe *et al.* 1982; Dixon 1983a). It is while cells are in the dividing state that the DNA is most susceptible to damage from mutagenic agents. It follows that the developing egg cells have the potential to accumulate genetic damage in their nucleus, a phenomenon which can be used to enhance the sensitivity of any method used to visualise chromosomal damage. Mussel eggs have been demonstrated previously to have the potential to accumulate organic contaminants and heavy metals, as well as some radionuclides (e.g. Rossi & Anderson 1977; Clifton *et al.* 1983).

The first sucessful attempt to apply the sister chromatid exchange (SCE) technique to mussel chromosomes was made by Harrison & Jones (1982). To ensure an adequate supply of dividing cells, these authors focussed on the early, soft-bodied larval stages (12–24 hours old), which they showed were sensitive to a variety of direct- and indirect-acting mutagens under acute exposure conditions (Harrison & Jones 1982; Dixon *et al.* 1985). At about the same time, Dixon & Clarke (1982) reported an attempt to apply the SCE technique to cells of adult mussels. The sensitivity of the mussel, and some other aquatic organisms (Kligerman 1979;

Pesch *et al.* 1981), to direct-acting mutagens (e.g. the alkylating agent mitomycin C) is similar to that of mammalian cells tested *in vivo*, which enhances the potential of these test systems for environmental monitoring (e.g. Dixon *et al.* 1982; Dixon 1983b). However, a major drawback with the use of the adult mussel for SCE studies is the lack of a readily identifiable meristematic tissue which can act as a source of dividing cells. Consequently, adult mussels are not in themselves suitable material for environmental monitoring purposes (De Flora *et al.* 1991). For this reason, those groups working with *Mytilus* have moved towards the scoring of micronuclei (MN) (e.g. Brunetti *et al.* 1988; Migiliore *et al.* 1990) which do not require cells to be in metaphase. Ideally, only binucleated (i.e. cytochalasin B-treated) cells should be scored for MN; this enables recently divided cells to be identified and reduces the likelihood of false positives stemming from artefacts and viral inclusion bodies (Hooftman & De Raat 1982).

Brunetti and co-workers were among the first to recognise the potential of the developing mussel egg as a system for monitoring genotoxic pollutants in the marine environment (Brunetti *et al.* 1986, 1989). Mussel eggs, because of their small size, allow experiments to be conducted in small volumes of water, thereby making considerable savings on expensive chemicals and apparatus. Brunetti's group showed that mussel embryos (8–32 cell stage) are sensitive to a range of water-borne mutagens, including mercuric chloride. Improvements in technique are described in a later paper (Brunetti *et al.* 1989). However, one potential drawback with their approach is that cells which have been through repeated cycles of DNA synthesis and repair, i.e. post-exposure to a mutagen, will not give a true indication of the primary level of genetic damage. The worst affected cells will have been lost through selective mortality which, when combined with the ameliorating influence of DNA repair, acts to drastically reduce the visible level of chromosomal damage. It follows that cells should be scored during the first few cell divisions after mutagen exposure (Stetka & Wolff 1976). For this reason, we focussed on 2-cell-stage embryos, which is the nearest it is possible to get to the original egg cell while still allowing for two cell cycles during which BrdU incorporation occurs (Fig. 1).

The source of pollution in the Red and Fal rivers is linked mainly with run-off from mine tailings which results in high copper and zinc levels in sediments and a variety of marine biota, including mussels (Bryan & Gibbs 1983). In addition there is contamination from chlorinated and petroleum hydrocarbons, plus moderately high levels of the antifouling agent tributyltin (TBT) near the mouth of the River Fal (chemical data supplied courtesy of South West Water and Dr Geoff Bryan, Plymouth Marine Laboratory).

Unlike nickel, mercury, lead, tin, arsenic, and chromium (not present at high levels at the study localities), zinc and copper are not believed to be mutagenic or carcinogenic (Friberg 1988). Studies of the behaviour of these two heavy metals in the tissues of marine organisms exposed to high levels in the laboratory and in the field have shown that the majority is sequestered in cellular organelles and is therefore rapidly detoxified; a small fraction remains metabolically available as these two metals are both essential elements in biological systems (reviewed in Phillips & Rainbow 1993). It is likely, therefore, that the correlation we observed between increased SCE levels and the high concentrations of zinc and copper in the Fal and Red rivers (Table 1) is not indicative of a direct cause-effect relationship, but instead relates to some other aspect of water quality at the different sites.

Dixon & Prosser (1986) demonstrated that TBT, the organotin antifouling compound present in the River Fal estuary, poses no direct genotoxic threat to marine life (i.e. mussels). There is some evidence, however, that hydrocarbons (e.g. from boating activity) can act synergistically with TBT to produce genotoxic damage (Dixon & McFadzen 1987). Since the River Fal is a major centre for boating activity, there is the possibility that it was these compounds and not

the high levels of heavy metals which caused the increase in SCE in mussel embryos. A different explanation is required, however, for the Red River where boating activity does not occur to any significant extent.

Since SCE has a complex aetiology, representing both DNA damage and repair (Latt 1974), the recorded level of SCE represents an integration of both these processes. Agents which are not mutagenic but which have the ability to disrupt DNA repair (Walton *et al.* 1983) have the potential to elicit a genotoxic response brought about by their reducing the efficiency of DNA repair. It is possible that the increase in SCE in mussel embryos originating from the Red River was not a direct genotoxic response but may instead reflect disturbance of some process connected with DNA repair. This aspect deserves further investigation. It is worth noting that cancer researchers already recognise the important role played by environmental influences and life-style as key factors influencing tumour initiation and promotion (e.g. Schribner & Suss 1978; Obe 1982).

Throughout the UK, *Mytilus edulis* reproduces in the spring, while some populations in the south of the country also release a second (smaller) batch of gametes in the autumn (Seed 1975). In view of this periodicity in availability of gametes in this species, great care has to be taken to ensure that the spawning peak is not missed when planning field investigations. In an attempt to overcome this problem (see also Brunetti *et al.* 1989), we have investigated the serpulid tubeworm *Pomatoceros lamarckii*, as an alternative test species for gentoxicity testing (Dixon 1985). In *Pomatoceros* (unlike *Mytilus*), ripe gametes are available virtually throughout the year (by artificial spawning), thus ensuring an almost continuous supply of embryos for cytogenetic analysis. An attempt is currently being made to link a cytogenetic end-point (i.e. chromosome aberrations) to a larval toxicity bioassay (ASTM 1990), in order to address the question of the consequences of genetic damage on offspring survival and viability.

Dedication

This article is dedicated to the memory of Dr Geoff Bryan, 1934-1993.

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