Aquatic Toxicology 159 (2015) 36–43

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Parental exposure to environmental concentrations of diuron leads to aneuploidy in embryos of the Pacific oyster, as evidenced by fluorescent in situ hybridization

Audrey Barranger[a,b,*], Abdellah Benabdelmouna[a,*], Lionel Dégremont[a], Thierry Burgeot[b], Farida Akcha[b]

[a] Ifremer, SG2M, Laboratory of Genetics and Pathology of Marine Molluscs, Avenue de Mus du Loup, 17390 La Tremblade, France
[b] Ifremer, Department of Biogeochemistry and Ecotoxicology, Laboratory of Ecotoxicology, Rue de l'Ile d'Yeu, BP 21105, 44311 Nantes Cedex 03, France

ARTICLE INFO

Article history:
Received 25 July 2014
Received in revised form 29 October 2014
Accepted 13 November 2014
Available online 24 November 2014

Keywords:
Aneuploidy
FISH
Diuron
Crassostrea gigas.

ABSTRACT

Changes in normal chromosome numbers (i.e. aneuploidy) due to abnormal chromosome segregation may arise either spontaneously or as a result of chemical/radiation exposure, particularly during cell division. Coastal ecosystems are continuously subjected to various contaminants originating from urban, industrial and agricultural activities. Genotoxicity is common to several families of major environmental pollutants, including pesticides, which therefore represent a potential important environmental hazard for marine organisms. A previous study demonstrated the vertical transmission of DNA damage by subjecting oyster genitors to short-term exposure to the herbicide diuron at environmental concentrations during gametogenesis. In this paper, Fluorescent in situ hybridization (FISH) was used to further characterize diuron-induced DNA damage at the chromosomal level. rDNA genes (5S and 18-5.8-28S), previously mapped onto *Crassostrea gigas* chromosomes 4, 5 and 10, were used as probes on the interphase nuclei of embryo preparations. Our results conclusively show higher aneuploidy (hypo- or hyperdiploidy) level in embryos from diuron-exposed genitors, with damage to the three studied chromosomal regions. This study suggests that sexually developing oysters are vulnerable to diuron exposure, incurring a negative impact on reproductive success and oyster recruitment.

1. Introduction

Cells characterized by the loss or gain of one or more whole chromosomes in comparison to the normal haploid or diploid condition are considered as aneuploid. Aneuploidy is the most common genetic defect in humans, and somatic aneuploidy has been linked to the development of various cancers (Kops et al., 2005; Weaver and Cleveland, 2006). Germinal aneuploidy can have major detrimental effects, as it impacts the following generation, resulting in mental retardation, congenital malformation and miscarriage. When all classes of numerical chromosomal abnormalities (monosomy, trisomy, polyploidy) are taken into account, these abnormalities are thought to contribute to more than half of all spontaneous abortions; aneuploidy is thus considered as the major cause of reproductive loss in man (Aardema et al., 1998; Abruzzo and Hassold, 1995; Hassold and Hunt, 2001).

Changes in normal chromosome numbers due to abnormal chromosome segregation may arise spontaneously (age, genetic instability, etc.), or as a result of chemical/radiation exposure, particularly during cell division. Several chemicals are known to induce aneuploidy *in vitro* or in animals *in vivo*. The marine environment and, in particular, coastal ecosystems are continuously subjected to various contaminants, originating from urban, industrial and agricultural activities. Certain contaminants exert their effects via genotoxic mechanisms, simultaneously causing embryotoxicity, carcinogenesis and long-term damage to organisms (Jha et al., 2000). Among these chemicals, phytosanitary products are a major concern in France, which is the leading user of agrochemicals in Europe and the third-biggest user worldwide (Jacquet et al., 2011; UIPP, 2012). During rain events, pesticides can be drained by catchment areas and contaminate coastal waters, hence creating an environmental risk for aquatic organisms. The bio-ecological particularities of marine bivalves (filter feeders, sessile mode of life and ability to bioaccumulate pollutants) make them particularly

* Corresponding authors. Tel.: +33 5 46 76 26 32; fax: +33 5 46 76 26 11.
E-mail addresses: audrey.barranger@ifremer.fr, audrey.barranger@gmail.com (A. Barranger), abdellah.benabdelmouna@ifremer.fr (A. Benabdelmouna).

http://dx.doi.org/10.1016/j.aquatox.2014.11.011
0166-445X/

sensitive to chemical stress, which can lead to reversible or irreversible genomic abnormalities such as DNA breaks or chromosomal aberrations. The relationship between genotoxicity and pollutants or polluted environments has been well investigated in marine invertebrates and, in particular, in oysters and mussels (Akcha et al., 2012; Bihari et al., 2003; Dallas et al., 2013; Marcheselli et al., 2011; Wessel et al., 2007). Moreover, the parental transmission of DNA damage has already been demonstrated, although the associated literature is incomplete (Barranger et al., 2014; Bouilly, 2004). The consequences of genomic abnormalities induced by chemicals on animal fitness are of major interest for understanding the long-term impact of genotoxic pollutants on wildlife.

Regarding the Pacific oyster, high mortality phenomena have been observed in summer in many regions of the world, in particular, on French coasts (Samain et al., 2007). In most cases, summer mortality is not allocated to a single factor, but a combination of several parameters, including physiological stress, environmental conditions and pathogenic organisms (Dégremont et al., 2010; Huvet et al., 2010; Renault et al., 1994; Samain et al., 2007). As many oyster production areas are subject to anthropic pressures, in particular, pesticides inputs (Burgeot et al., 2008; Munaron et al., 2003), the possible role of environmental pollution in oyster mortality events is being increasingly examined in France and elsewhere (Ochoa et al., 2012). Understanding this phenomenon is a complex but very important challenge, as the Pacific oyster, *Crassostrea gigas*, is one of the foremost aquaculture resources worldwide (FAO, 2012) and France is currently Europe's leading producer. This species, whose diploid chromosome number is 20 ($2n = 2x = 20$ chromosomes), can tolerate a genome size variation of between 5 and 15% (DNA aneuploidy) and a percentage of hypodiploid cells with 17, 18 or 19 chromosomes varying from 5 to over 30% (Benabdelmouna et al., 2011; Leitão et al., 2001b; Thiriot-Quiévreux et al., 1992; Zouros et al., 1996). Various methods are available for characterizing these genomic abnormalities. Originally, cytogenetic analysis could only be performed by conventional karyotyping. However, this approach is technically demanding, time-consuming and limited by the number of metaphase cells available for analysis. Additionally, poor chromosome preparations and frequent artifactual variations in chromosome numbers may compromise results. These limitations can be overcome by recent molecular cytogenetic techniques such as fluorescent in situ hybridization (FISH), allowing chromosome and gene identification, not only onto metaphase preparations, but also starting from the more accessible material represented by interphase nuclei (I-FISH). This method has essentially been used in higher vertebrates, such as human, equine, bovine and porcine cells (examples in embryo nuclei: Rambags et al., 2005; Viuff et al., 2002; Zudova et al., 2003). FISH analysis has already been used for the chromosome characterization and identification of bivalves (Benabdelmouna et al., 2008; Cross et al., 2005; Wang et al., 2004; Xu et al., 2001), but never previously for the detection of genomic abnormalities in ecotoxicological studies.

In a previous experiment, the vertical transmission of DNA damage was highlighted by exposing oyster genitors to short-term environmental concentrations of diuron—a substituted urea herbicide used for on-land weed control—during gametogenesis (Barranger et al., 2014). In spat from diuron-exposed genitors, a significant decrease in nuclear DNA content was measured, with over 15% of individuals showing DNA hypodiploidy, i.e. a decrease in their genome size relatively to eudiploid oysters.

In this study, rDNA probes were hybridized on interphase nuclei (I-FISH) from embryos produced by controls and diuron-exposed oyster genitors in order to supplement information on the aneugenic effects of diuron. These genes were chosen to specifically label particular chromosomes and identify their numerical variations. The major (18-5.8-28S) and minor (5S) rRNA genes used in our study are two families of ribosomal RNA genes in higher eukaryotes. 5S ribosomal RNA is the smallest RNA component of the ribosome. Both genes are organized into clusters of tandem repeats with up to hundreds or thousands of units (Martins and Galetti, 2001). In view of their high copy number, rRNA genes can easily be detected by FISH and used for chromosome identification (Fontana et al., 2003; Insua et al., 2001; Liu et al., 2003).

There are a number of advantages to studying freshly fertilized embryos. Firstly, the cells are in an active state of division; this is an ideal state for studying aneugenic effects leading to chromosome loss or gain, allowing adverse effects to be observed prior to cell death or removal of the aberration through DNA repair. Moreover, the study of early life-history stages provides various information on population fitness.

2. Materials and methods

2.1. Genitor origin, diuron exposure and fertilization

The adult Pacific oysters (*C. gigas*) used for this experiment were the progenies of wild oysters sampled in the Marennes-Oléron Bay (France). One hundred oysters were spawned in February 2011 at the Ifremer hatchery, to produce 6 batches, each cross using around 16 parents. These six batches were then mixed at the juvenile stage to get one group, in order to limit any batch effect. The resulting group of oysters was used for this experiment.

Oyster husbandry, diuron exposure and fertilization were performed as described in Barranger et al. (2014). Briefly, following a 1-month acclimatization period, sea water temperature ($8 \pm 1\,^{\circ}C$) was gradually raised by two degrees per day for 1 week, to reach $19.8\,^{\circ}C$ ($\pm 0.3\,^{\circ}C$). Once gonad development had begun, the oysters were divided into three experimental groups: a seawater control, a solvent control and a diuron-exposed group. Diuron—the pesticide selected for our study—is a substituted urea herbicide used in agriculture for on-land weed control. This herbicide is also used as an antifouling biocide (Thomas et al., 2001). In France, it has been banned as phytosanitary product since 2008 (JO no. 204 September 4, 2007), and as biocide used in antifouling paints since 2009 (bylaw 28 August, 2008). Although data on pesticide transfer from the continent to French coastal ecosystems is fairly rare, recent studies have reported the presence of diuron in various French aquatic environments (Atlantic bays, estuaries and Mediterranean Sea) (Buisson et al., 2008; Caquet et al., 2013; Munaron et al., 2012, 2006). As diuron stock solution is sold in acetonitrile, the solvent control group was exposed to acetonitrile at a concentration of 0.005%. This concentration of acetonitrile has already been used in a previous study of Akcha et al. (2012) and did not reveal any genotoxic and embryotoxic effects on spermatozoa or embryo, respectively. Three 250-L tanks were used for each experimental group, containing each 240 oysters. Two 7-day exposure periods took place at the start and mid-course of gametogenesis, during which the oysters were exposed to 0.4 and 0.6 μg/L of diuron, respectively. These short-exposure pulses were chosen to mimic rain events with concentrations of diuron close to those detected in coastal waters (see Barranger et al., 2014 for details).

Within the experimental group, when oysters were ripe, males and females ($n = 70$ oysters per group) were individually induced to spawn through thermal shocks (from 18 to $28\,^{\circ}C$ for 30 min). The oocytes were then pooled within group, as well as the spermatozoa, and the fertilization was achieved using 9,000,000 oocytes and a ratio of 200 spermatozoa per oocyte.