Marine Pollution Bulletin 62 (2011) 1437–1445

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Effects of chronic exposure to dispersed oil on selected reproductive processes in adult blue mussels (*Mytilus edulis*) and the consequences for the early life stages of their larvae

Thierry Baussant [a,*], Maren Ortiz-Zarragoitia [b], Miren P. Cajaraville [b], Renée Katrin Bechmann [a], Ingrid Christina Taban [a], Steinar Sanni [a,c]

[a] International Research Institute of Stavanger/IRIS, Mekjarvik 12, 4070 Randaberg, Norway
[b] Laboratory of Cell Biology & Histology, Dept. Zoology and Cell Biology, University of the Basque Country, P.O. Box 644, E-48080 Bilbao, Basque Country, Spain
[c] University of Stavanger, Faculty of Science and Technology, N-4036 Stavanger, Norway

ARTICLE INFO

Keywords:
Dispersed crude oil
Mytilus edulis
Parental exposure
Reproductive biomarkers
Early life stages

ABSTRACT

Mussels (*Mytilus edulis*) were continuously exposed to dispersed crude oil (0.015–0.25 mg/l) for 7 months covering the whole gamete development cycle. After 1 month exposure to 0.25 mg oil/l, the level of alkali-labile phosphates (ALP) and the volume density of atretic oocytes in female gonads were higher than those in the gonads of control females, indicating that oil affected the level of vitellogenin-like proteins and gamete development. Spawning of mussels was induced after 7 months oil exposure. Parental oil exposure did not affect subsequent fertilization success in clean seawater but this was reduced in 0.25 mg oil/l. Parental exposure to 0.25 mg oil/l caused both slow development and a higher percentage of abnormalities in D-shell larvae 2 days post-fertilization; reduced growth 7 days post-fertilization. These effects were greatly enhanced when larval stages were maintained at 0.25 mg oil/l. Similar studies are warranted for risk assessment prognosis.

1. Introduction

Discharges of produced water (PW) from offshore oil and gas operations have received increased attention in recent years due to their potential harmful environmental effects (Utvik and Gärtner, 2006). Produced water (PW) is the waste generated in the largest volume during production of oil and gas from offshore oil and gas wells (Neff et al., 2006). The chemical composition of PW is complex, including dispersed oil, dissolved hydrocarbons, organic acids, phenols, metals, and traces of chemicals added in the separation and production line (Utvik, 1999). The regulatory limit for total petroleum hydrocarbons in PW discharged offshore in the Norwegian sector of the North Sea is 30 mg/l. Oil and gas industries are required to monitor contaminants and potential effects of PW. In the Norwegian water column monitoring program, both blue mussels (*Mytilus edulis*) and cod (*Gadus morhua*) are typically being used for this purpose in cages deployed in the field along a gradient from an oil production site (Durell et al., 2006; Hylland et al., 2008; Sundt et al., 2009).

Mussels are used worldwide as key marine sentinel organisms (Cajaraville et al., 2000; Goldberg, 1975) because they can bioaccumulate high concentrations of hydrophobic compounds (e.g. polyaromatic hydrocarbons, PAHs) in their tissues (Baumard et al., 1998), rendering them particularly suited for the study of the biological effects of pollutants. The consequences of biological responses measured in indigenous or caged mussels from contaminated sites for effects at higher levels of biological organization need to be documented in order to judge the relevancy of field measurements for routine monitoring, decision-making and risk-based modeling. The link between early biological responses and subsequent ecologically relevant effects can help to better define the environmental risk of PW. However, these data are difficult to obtain in the field although bivalve larvae have been deployed in the field to study effects at contaminated sites such as harbors (Quiniou et al., 2007). Laboratory studies have limitations when attempting to simulate field conditions but can be used as a surrogate for field studies.

In a previous paper, Baussant et al. (2009) have reported on a long-term laboratory experiment with adult mussels continuously exposed to a dispersed North Sea crude oil: environmentally realistic concentrations of the mechanically dispersed North Sea crude oil were tested to simulate the continuous regular discharges of produced water in the field and tissue concentrations of PAHs were related to biomarker responses analyzed after 1 month. The mussels from this experiment were further exposed to the same concentrations of oil during the whole period of gametogenesis. The main objectives of the present paper are to study the subsequent effects of long-term parental oil exposure on the early life stage development of their larvae, and to investigate if a link between

* Corresponding author. Tel.: +47 51 87 55 03; fax: +47 51 87 55 40.
E-mail addresses: thierry.baussant@iris.no, tb@iris.no (T. Baussant).

0025-326X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2011.04.029

larval success and impairment of adult mussel gametogenesis can be established.

The effects of oil on reproduction in adult mussels were assessed by examining gonad histopathology, gamete stage development using a gonad index and the levels of vitellogenin-like proteins. The changes occurring in the gonads may affect gamete quality, and both larval development and survival with potential ecological consequences (Lewis and Galloway, 2009). Hence, additionally to the reproduction study in adult mussels, the development of early life stages was studied after spawning of the individuals. The assessment of marine pollution using early life stage tests provides an integrated biological response with high ecological relevance for the risk assessment process (His et al., 2000; Wessel et al., 2007; Bellas et al., 2008). To our knowledge, the present study is alone in reporting on the subsequent fate of early life stages after parental crude oil exposure in bivalves.

This work was part of the BioSea I joint industry research program where laboratory experiments with fish and invertebrates were carried out to implement and evaluate a suite of biomarkers for their application in offshore oil and gas monitoring programs in the North Sea and in the Barents Sea (Baussant et al., 2009; Bechmann et al., 2010; Larsen et al., 2006; Skadsheim et al., 2009; Taban et al., 2004).

2. Materials and methods

2.1. Experimental animals

Blue mussels, *M. edulis*, were collected in December 2002 at a shell farm located close to the Lysefjord (Rogaland county, Norway; supplier: Aspøy Skjell & Produktutvikling AS). After collection, they were transported in polystyrene boxes and placed in seawater again immediately on arrival at the research center facility (Akvamiljø AS, Randaberg, Norway). They were then transferred to a 350 l tank with running seawater (salinity of 34 ± 0.5 psu and temperature of 7.5 ± 0.5 °C. The seawater was pumped directly from a depth of 75 m from the fjord adjacent to the laboratory (Byfjord) and sand-filtered before use in the experimental system. During the two week pre-exposure maintenance period, mussels were fed regularly a mixture of live *Isochrysis galbana*, *Rhodomonas baltica* and *Skeletonema costatum*. No mortality was recorded during that period.

2.2. Oil exposure system

The continuous flow system described by Sanni et al. (1998) was used with some modifications to create a dispersion of oil in seawater. North Sea crude oil was placed at the top of a two-compartment glass cylinder separated by a Teflon® piston. At the bottom of the cylinder, distilled water pressurized by a HPLC pump, was used to push the piston upwards at the desired speed to deliver oil at the same rate into the continuous flow of seawater being fed to a header tank. The dispersion of oil was mechanically created using a mixing valve placed upstream from the oil injection point, allowing the fragmentation of oil to droplets of a mean size of 10 μm (see Baussant et al., 2009, for more details). Thereafter, dilution of the dispersed oil in the header tank with running seawater generated three nominal North Sea oil concentrations: 0.015 mg/l, 0.06 mg/l and 0.25 mg/l. Baussant et al. (2009) reported these nominal oil concentrations to correspond to actual total PAH concentrations ranging from 0.2 (low exposure) to 4 (high exposure) μg/l in the experimental system.

All treatments with mussels were carried out in 60 l glass aquaria with the flow rate maintained at ca. 800 ml/min. Approximately 500 individuals were placed into each of the control and exposure tanks at the onset of the exposure.

2.3. Experimental design and embryo-larval development

An overview of the experimental design is presented in Fig. 1A. Adult mussels were exposed to dispersed oil for up to 7 months using the continuous flow exposure system described in Section 2.2. During the first month of the experiment the temperature was on average 7.5 °C and increased gradually to 9.5 °C at the end of the experiment as seasonal warming progressed. The general assay methodology used for early life stages was based on that of His et al. (2000) and Quiniou et al. (2005). After 7 months oil exposure, mussels from each treatment (including control) were induced to spawn by rapid changes of seawater temperature from 9 °C to 20 °C. In addition algae were added to help spawning. For most treatments, mussels started to spawn after no longer than 1 h but spawning of the mussels from the 0.25 mg/l treatment was obtained after a longer period (2 h). A total of six spawning individuals of both genders were used to produce a pooled sperm solution and a pooled egg solution. Before use, the sperm was filtrated through a 30 μm nylon sieve and eggs were filtrated through a 100 μm nylon sieve to remove gross material.

Fertilization of eggs and sperm from adult mussels exposed to 0.015, 0.06 and 0.25 mg oil/l was performed in clean seawater to study effects of parental exposure alone. The control batch was used as the reference for comparison. For all treatments, fertilization was obtained by cautiously transferring the pooled egg solution into a 2 l flask filled with filtrated seawater at 15 °C, then adding ca. 5 ml of the pooled dense sperm solution. Sperm mobility was checked before addition into the flask. Two additional treatments were included in the larvae assay; (1) eggs and sperm from mussels exposed to 0.25 mg oil/l were maintained in 0.25 mg oil/l (combined effects of parental and direct seawater exposure to oil on embryo-larval development) and (2) eggs and sperm from control mussels were transferred to 0.25 mg oil/l (direct effects of oil exposure on embryo-larval development). For these two last treatments, fertilization was performed in a 2 l flask filled with 0.25 mg oil/l at 15 °C.

Fertilization success (visualized by expelled polar bodies) was recorded within the first 2 h following the mixing of gametes and prior to the transfer into the experimental flasks. Careful manual agitation was regularly maintained during that period to keep eggs in suspension. Two to three sub-samples were redrawn from the 2 l egg solution and the number of fertilized eggs was counted using light microscopy (×400). Further, the eggs were transferred to a continuous flow system consisting of a series of 1 l Duran glass bottles placed in a climate room regulated at 15 °C. The lid of each bottle was modified to obtain flow-through circulation of the water (10 ml/min) and the outlet was equipped with a 40 μm nylon mesh (Fig. 1B). Approximately 20 eggs/ml were added to each bottle at the onset of the experiment. Four replicates were used for each treatment group. The water used was either control seawater (for control and parental exposure scenarios) and North Sea oil exposed seawater at 0.25 mg/l for the two additional treatments. Seawater temperature in the larvae system was adjusted to 15 °C.

The larvae were maintained in the experimental system for a period of 21 days when umbo larval stages were observed. No food was provided during the first two days after transfer to the larvae experimental system. Thereafter, a peristaltic pump was used to deliver a continuous flow of the algae *I. galbana* adjusted to 40,000 cells/ml to each replicate bottle.

Sampling for embryo-larval development determination was made at 2, 7 and 21 days post-fertilization. At each sampling, the content of each experimental bottle was carefully poured into a nylon strainer with a 40 μm mesh, the larvae were carefully