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Valve gape behaviour of mussels (*Mytilus edulis*) exposed to dispersed crude oil as an environmental monitoring endpoint

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ABSTRACT

Environmental monitoring requires cost-effective and efficient methods for detecting potential effects of pollution, and valve gape behaviour has been used with this purpose for a range of contaminants in freshwater and marine bivalves. The current study investigated the use of a new method for measuring valve behaviour responses in mussels (*Mytilus edulis*) exposed to dispersed crude oil (DCO). Results confirmed that valve gape is a sensitive parameter; at the high DCO concentration (0.25 mg L^{-1}) the mean valve gape was reduced from 49 to 31%, and mussels increased shell movement (measured as distance travelled) or spent more time closed to avoid contact with the oil. At the low DCO concentration (0.015 mg L^{-1}) the distance travelled parameter was the most sensitive endpoint. Results also demonstrated that valve gape behaviour is a valid endpoint when monitoring mussels for exposure to DCO.

1. Introduction

Oil and gas activity operations are one of many potential pollution sources in the marine environment. Regarding oil contamination, there are many sources of oil in seawater, including but not limited to natural oil seeps, accidental spills and discharges from human activities (Pampanin and Sydnes, 2013). Cost-effective and efficient procedures for monitoring the environment and forecasting the potential impacts of pollutants like oil are important to preserve the ecosystem integrity.

In this context, bivalve molluscs have been successfully used as bioindicators in environmental monitoring (Goldberg, 1986; Martin and Richardson, 1995; Flynn et al., 2013; Shue et al., 2014), due to their sessile and filter-feeding habits, which lead to bioaccumulation of pollutants, generally reflecting the near surrounding environment (Burns and Smith, 1981). The use of biological measurements (biomarkers) in bivalves is well-established (Depledge and Fossi, 1994; Hyne and Maher, 2003), and the use of behavioural endpoints is highly desired for their high ecological relevance (Gerhardt et al., 2005). Valve movement measurement is particularly useful as this technique can be applied to a wide range of bivalves living in different biotopes, allowing the use of naturally occurring species (Liber et al., 2007). In natural conditions, bivalves are known to hold shell valves open for long periods of time in order to facilitate respiration and feeding processes. Valve closure can occur under periods of physiological stress (Kramer et al., 1989). Shell movement has been widely used in monitoring of acute changes in the environment, recently receiving attention as a relevant ecotoxicological parameter (Hartmann et al., 2016). The ecological relevance of valve movement behaviour is related to the filtration rate (Riisgård and Larsen, 1995; Jørgensen, 1996), which is linked to both growth and reproduction (Widdows et al., 1997). Several endpoints have been suggested when utilising valve gape (VG) in contaminant exposure, including the number of individuals in a closed position and/or the amount of time spent in open or closed positions (Kramer et al., 1989; de Zwart et al., 1995), avoidance behaviour (fully closed shell), transition frequency as an indicator of increased movement between open and closed (Hartmann et al., 2016), and the velocity of valve movement (Ahmed et al., 2015). VG is useful in biomonitoring predominantly as an indicator of short-term changes in environmental conditions (Kramer et al., 1989). VG is a sub-lethal response, and it is a non-destructive measurement that can be followed over long periods of time. This type of response can be 10–100 times more sensitive to exposure than traditional mortality endpoints (Robinson, 2009; Hasenbein et al., 2015). Moreover, VG data can be extrapolated to feeding behaviour and energy use, and can thereby act as a warning system for changes at ecosystem level. VG can be measured in the field and in real-time, meeting the need for in situ effects measures (Liber et al., 2007). Recently, Bamber and Westerlund (2016) have suggested that the shell

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movement, measured as total distance of valve movement, may be particularly useful when examining data collected over a long time period. Most previous scientific work has focused on the effects of chemicals, including heavy metals (Davenport and Manley, 1978; Slooff et al., 1983; Doherty et al., 1987; Kramer et al., 1989; Sluyts et al., 1996; Curtis et al., 2000; Tran et al., 2003; Liao et al., 2005; Fdil et al., 2006; Liao et al., 2009; Chen et al., 2010; Ayad et al., 2011; Beyer et al., 2013; Hartmann et al., 2016; Jou et al., 2016) or natural environmental changes (e.g. food availability, temperature) and natural conditions (Loosanoff, 1939, 1942; Higgins, 1980; Ortmann and Grieshaber, 2003; García-March et al., 2008; Gnyubkin, 2010; Robson et al., 2010; Haberkorn et al., 2011; Jou et al., 2013; Riisgård and Larsen, 2014; Ahmed et al., 2015; Garcia-March et al., 2016), in a wide range of species. However, few studies are published showing the effect of dispersed crude oil on valve activity (Kramer et al., 1989; de Zwart et al., 1995).

Valve movement has been measured using different approaches. The first publication of real-time measurements of VG was published by Marceau (1909), where movements were recorded by mechanical means. An early method was developed to measure VG indirectly by measuring qualitative changes in the water flow out of the exhalent siphon (reviewed by Salánki et al., 1991). Three direct valve movement measurement methods are now available; 1) an electromagnetic system (Schuring and Geense, 1972 in Kramer and Botterweg, 1991 and described in further detail by Kramer et al., 1989), 2) a Hall sensor and magnet system calibrated to record gape angle (Wilson et al., 2005; Robson et al., 2006), and 3) a method involving light emission changes (Frank et al., 2007). A few commercially available systems incorporate VG measurement, including the Mosselmonitor (de Zwart et al., 1995) and the Dreissena-Monitor (Borcherding, 1992). All of these methods require some form of instrumentation of the organisms, by fixing one or more components of the monitoring system onto the shells. New methods that are non-intrusive would contribute to reducing the possibility of artefact effects due to the attached instrumentation, and reduce maintenance requirements as a result of instrumentation loosening.

The first objective of this work was the introduction of a non-intrusive VG monitoring technique that utilises a laser triangulation sensor to measure VG from a distance. The second objective was to document the effect of dispersed crude oil (DCO) on valve movement. VG behaviour was monitored in mussels exposed to three sub-lethal concentrations of DCO (0.015 mg L^{-1}, 0.06 mg L^{-1} and 0.25 mg L^{-1}) and two valve movement endpoints were examined: the extent of VG and the distance travelled (DT).

2. Materials and methods

2.1. Animal collection

Mussels (*Mytilus edulis*) were collected at Kvitsøy (south west Norway) and transported to the IRIS-Environment laboratory facility (Randaberg, Norway). Mussels were then transferred into 50 L tanks with flowing seawater.

2.2. Animal feeding

During maintenance and experimental periods, mussels were fed with microalgae concentrate containing a mixture of *Isochrysis* spp., *Pavlova* spp., *Thalassiosira wiessflogii*, and *Tetraselmis* spp. (Shellfish Diet 1800, Instant Algae, Reed Mariculture Inc., USA). The mixing of microalgae concentrate with filtered seawater in the algae line is illustrated in the supplementary material (S1). The microalgae concentrate was diluted to 8 million cells per mL and added to filtered seawater in the main header tank using a peristaltic pump at 8.75 mL min^{-1}, giving a nominal algal concentration of 15,000 cells mL^{-1}.

2.3. Environmental conditions

Water temperature was controlled throughout the experiment to 13 °C and monitored daily using a Pyroscience submersible temperature sensor (TSUB21) attached to a FireStingO2 sensor (Pyroscience, Aachen, Germany). The seawater had a salinity of 32.5 ppt.

To control particle concentrations in the header tank, samples were taken daily and measured using a Coulter counter (Multisizer 3, Beckman Coulter, USA). Three water samples (2 L each) were taken from the header tank for particulate organic and inorganic matter (POM/PIM) analyses. The seawater samples were filtered on pre-ashed and tared GF/C filters (47 mm; Whatman International Ltd., UK) and frozen at −80 °C. Filters were dried at 60 °C to constant weight and reweighed, giving the total particulate matter (TPM). The filters were ashed afterwards at 450 °C for 4 h, and reweighed to obtain the PIM. The difference between TPM and PIM was the POM content of the seawater sample.

2.4. Exposure set up

Each exposure scenario was carried out over the course of one week. Experiments were carried out on six mussels at a time, in individual flow-through chambers measuring 155 × 100 × 85 mm (length × width × depth), and with a total volume of 1.2 L (Fig. 1). The mean water flow rate was 200 mL min^{-1} (±10 mL min^{-1}). One mussel of a similar size (Table 1) was fixed in each chamber by gluing it to an epoxy mould. The glue (Pattex PL700) is solvent free. Mussels were given several hours to acclimate before the measurement was started, and all mussels attached themselves to the surfaces of the chamber with byssal threads.

Mussels were forced to close with gentle prodding in order to achieve a zero gape setting on the triangulation sensor (SICK OD Precision Displacement Sensors, SICK Sensor Intelligence, Germany). The SICK sensor was positioned visually so that the laser beam focussed on the edge of the shell. The VG was measured as the distance to the mussel every 2 s for the entire experimental period.

The SICK sensor can generate error messages in certain situations. Error messages were treated according to standard SICK procedure, by applying the last valid distance measurement. The causes of errors and their implication for the data are presented in the result and discussion sections.

2.5. Pre-exposure study

A pre-exposure study was carried out to test the experimental set up and to measure the range of expected behaviour in unexposed organisms. Six mussels were maintained in clean seawater for three days. Mussels were fed according to the conditions described in the section on animal maintenance and VG was measured.

2.6. Oil exposure study

Measurements of VG were carried out in clean seawater for three days, before the exposure started. Mussels were then exposed to DCO for four days.

A light, low sulphur, North Sea crude oil was dispersed under pressure using a continuous flow system (CFS) as described by Sanni et al. (1998). The CFS system produced seawater with an oil concentration of 5 ppm, and DCO droplets had a median size of 10 μm (measured on the Coulter counter). The DCO in the oil header tank was mixed with seawater and algae from the main header tank to generate the three nominal exposure concentrations: 0.015 mg L^{-1} (low), 0.06 mg L^{-1} (medium) and 0.25 mg L^{-1} (high). The oil line is illustrated in the Supplementary material (S1).

At the end of each exposure scenario, mussels were removed from the chambers and stored frozen at −20 °C for PAH analysis.

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