Report for MASTS Small Grant OGSG3

Effect of hydrocarbons and dispersants on model shallow-water sponges *Halichondria panicea*

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Introduction

Sponges (phylum Porifera) are key organisms in aquatic environments. Sponges provide a wide array of ecosystem services in the marine environment they are present in (Bell *et al.* 2008). Marine sponges contribute to the recycling of vital nutrients such as carbon, nitrogen and silica (Maldonado *et al.* 2005; Maldonado *et al.* 2011). Indeed, sponges can efficiently filter large volumes of seawater and retain Dissolved Organic matter (DOM) (Yahel *et al.* 2003, De Goeij *et al.* 2008). By releasing cell detritus into the water column, they transform DOM to Particulate Organic Matter, making carbon readily accessible to other taxa, in a process known as the 'sponge loop' (De Goeij *et al.* 2013, Alexander *et al.* 2014, Maldonado 2016). Furthermore, many bacterial micro-organisms associated with the sponges have been found to play a role in different parts of nitrogen cycling through their nitrification, denitrification or AMMONOX capacities (Hoffman *et al.* 2009). Through the sponge loop and the recycling of both carbon and nitrogen, sponges are considered to participate into the bentho-pelagic coupling (Bell et al. 2008, Maldonado *et al.* 2016).

As anthropogenic pressures on marine environments such as ocean acidification, global warming and pollution are growing, understanding the impacts of human activities on key marine organisms such as sponges is becoming increasingly important. Evidence for the capacities of sponges to bioaccumulate low concentration of pollutants such as dissolved metals and poly aromatic hydrocarbons has been growing over the last 16 years (Glyzina *et al.* 2002, Cebrian *et al.* 2003, Berthet *et al.* 2005, Rao *et al.* 2006, Mahaut *et al.* 2013, Batista *et al.* 2013, Gentric *et al.* 2016). More recently evidence of the molecular impact of exposure to pollutants including hydrocarbons and heavy metals with the activation of signalling pathways involved in the response to oxidative stress has also been showed in sponge tissue (Châtel *et al.* 2011). However, to the authors knowledge no study investigating the physiological impacts of a pollutant such as crude oil at high concentration has been carried out.

The objectives of this study for which the MASTS Oil and Gas forum small grant OGSG3 was allocated was: (1) to investigate the physiological impacts of crude oil and/or dispersant

contaminated seawater on sponge *Halichondria panicea* and (2) characterise the effects of exposure to crude oil and/or dispersants contaminated seawater on the transcriptome of the sponge *H. panicea*.

Material and methods

Sample collection and preparation of treatment solutions

Sponge sampling for the purpose of this study was conducted at low tide at Coldingham bay, about 75 km southeast of Edinburgh. Seawater was also collected from this site to prepare the treatment solutions for the experiment.

After collection, the samples were returned to Heriot-Watt University. Sponges were kept in retention tanks at 10°C for 48h until the beginning of the experiment. Seawater was used to produced Water Accommodated oil Fraction (WAF) and Chemically Enhanced Accommodated oil Fraction (CEWAF) as described by the CROSERF protocol (Aurand and Coelho, 2005). In short, oil (Schiehallion crude oil) and/or dispersants (Slickgone NS) were mixed for 48h prior to the start of the exposure experiment with seawater to produce treatment solutions for the oil (WAF) and oil and dispersant (CEWAF) experimental conditions (Fig. 1A). Additionally, three other conditions were tested in this study: control seawater (without any contaminants), benzo-a-pyrene in DMSO (BaP) contaminated seawater and DMSO in seawater.

Figure 1 Experimental set-up (A) WAF and CEWAF preparations (B) Flow through set-up (C) Peristatltic pump (D) Individual incubation chamber

Experimental set-up

In order to safely expose samples of *H. panicea* to the treatment conditions described above, a flow-through experimental design was used (Fig. 1B). Fifteen samples of *H. panicea* were placed in 750 mL individual glass incubation chambers. Sponges were randomly allocated to the treatments (3 replicates per treatment) and PTFE inflow lines connected the chambers to two 6-channels Marine Colors peristaltic pumps set to provide a flow of 750mL/24h. PTFE outflow tubes enable the excess water to escape the chambers and to be collected in containers for appropriate disposal (Fig. 1C). Individual magnetic stirrer present in each chamber gave good water mixing in the chamber (Fig. 1D). Sponges were allowed to acclimatise in the chambers for 48h before exposure. Sponges were then exposed to the appropriate treatment for 48h. After exposure, sponges were also kept for another 48h in the chambers and gradually provided with uncontaminated seawater.

Respiration rate and algal clearance rate of each sponge were measured before, during and after exposure to assay baseline metabolism and feeding efficiency respectively. Oxygen concentration in the seawater surrounding the sponge was measured using the Presens Oxy-4 optode system. Respiration blanks were also measured for each treatment to assess microbial respiration in the seawater. Sponge respiration rate was determined as follows:

$$Respiration\ rate = (Resp_{Chamber} - Resp_{blank})/V_{sponge}$$

where $Resp_{chamber}$ is the respiration rate determined in the chamber with a sponge, $Resp_{blank}$ is the respiration rate determined in the blank chamber without a sponge and V_{sponge} the volume of sponge tissue (determined at the end of the experiment).

To determine clearance rate, a concentrated algal solution (Reed Mariculture Shellfish Diet 1800™ composed of *Isochrysis spp*, *Pavlova spp*, *Tetraselmis spp*, *Thalassiosira pseudonana*, *Thalassiosira weissfloggi* and *Chaetoceros spp*) was added to each chamber and the sponge were left to filter for 2h. Water samples were collected every 20mins and algal cell concentrations were determined by measuring samples total absorbance. A calibration curve was previously established relating total absorbance to algal cell densities determined by cell count under light microscopy. Clearance rates for each sample was calculated as described by de Goeij *et al.* 2008, as follows:

$$Clearance\ rate = ((\left(\frac{V_{water}}{t}\right)\ln\left(\frac{C_0}{C_t}\right))/V_{sponge}$$

where V_{water} is the volume of water in the chambers, t the time of incubation, C_0 and C_t the initial and final concentration of algae in the chamber and V_{sponge} the volume of sponge tissue.

At the end of the experiment, tissue samples were collected in preserved in RNA later for further RNA extraction and transcriptomic sequencing. RNA extraction were conducted with a QIAGEN Total RNA tissue extraction kit and transcriptomic sequence of mRNA was provided by Edinburgh Genomics.

Statistical analysis and transcriptomic data analysis

Analysis of Variance (ANOVA) were performed to test for statistical difference in clearance rate and respiration rate respectively between treatment and time points. Statistical analysis of the data was conducted using the software RStudio (R core team 2015).

After reception of the sequenced data, Illumina adaptor sequences were removed from the reads with Cutadapt (Martin 2011). Reads quality was then assessed using FastQC (Andrew 2010).

Results and discussion

Physiological impacts of exposure to contaminated seawater

Overall, respiration rate measured during this experiment varied between 0.59 and 35.34 µmol cm^{-3} $hour^{-1}$. High variability between individual sponges and between time points can be observed (Fig. 2A). However, no clear difference between treatments could be detected. These measurements are, nevertheless, in accordance with the high natural variability in sponge metabolic rate recorded in the wider literature (e.g. Osinga *et al.* 1999).

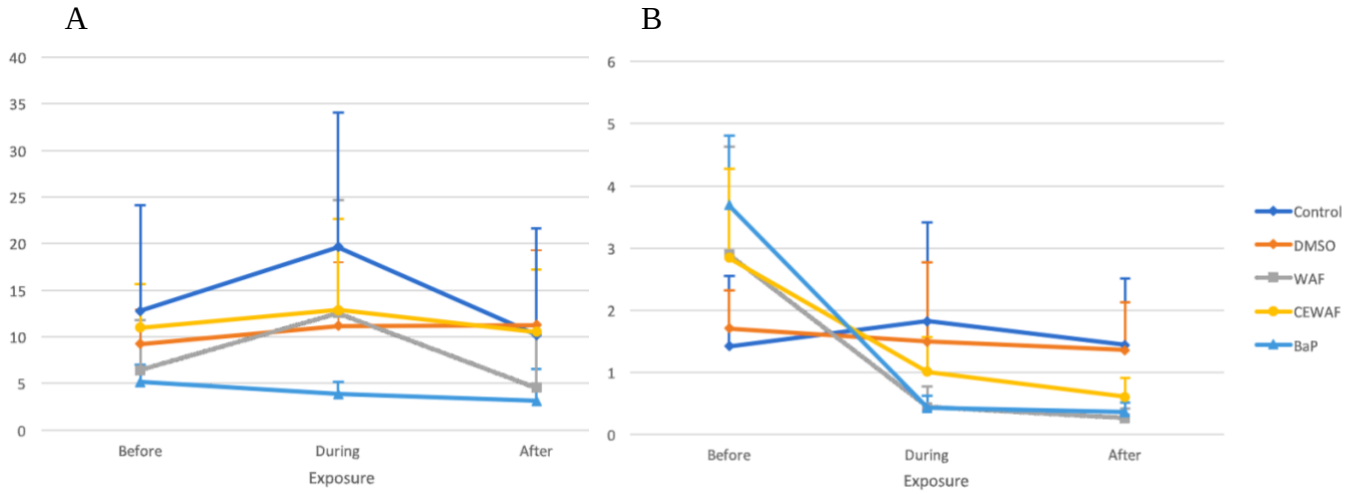

Figure 2 Pysiological measurements (A) Respiration rate in µmol of O_2 consummed per cm^3 of tissue per hour for each treatment before, during and after exposure (B) Clearance rate in cm^3 of water cleared per cm^3 of tissue per minute for each treatment before, during and after exposure.

Overall clearance rate measured during this experiment varied between 0.11 and 4.89 cm^3 cm^{-3} min^{-1} (Fig. 2B), which is within the range described in the literature for *H. panicea*

(De Goeij *et al.* 2008). High variability between individual clearance rates can still be observed but a strong and statistically significant decrease in clearance rate was detected in the WAF, CEWAF and BaP treatment during and after exposure (Fig. 2B). Clearance rate measure the amount of water filtered by the sponge in time and so can be used as a proxy for filtration rate. Here, we hence show that the filtration behaviour of sponge *H. panicea* is diminished during exposure to hydrocarbon contaminated seawater and that filtration behaviour does not resume even 48h after the end if the exposure.

Early stage results of transcriptomic study

Overall sequencing yielded very good results as all samples provided over 15 million reads (table 1).

Treatment	***Average number of reads***	***Number of low quality reads***	***Read length***	***Average %GC***
Control	*16 608 334*	*0*	*50-75*	*47*
DMSO	*19 035 601*	*0*	*50-75*	*47*
WAF	*19 923 358*	*0*	*50-75*	*47*
CEWAF	*18 127 218*	*0*	*50-75*	*48*
BaP	*22 131 019*	*0*	*50-75*	*48*

Table 1 Overview of data received after Illumina Sequencing at Edinburgh Genomics

Read length, after adaptor trimming, vary between 50 and 75bp (table 1). No low quality reads were detected in the samples and phred scores for each samples were very high (above 30) as can be seen in Fig. 3.

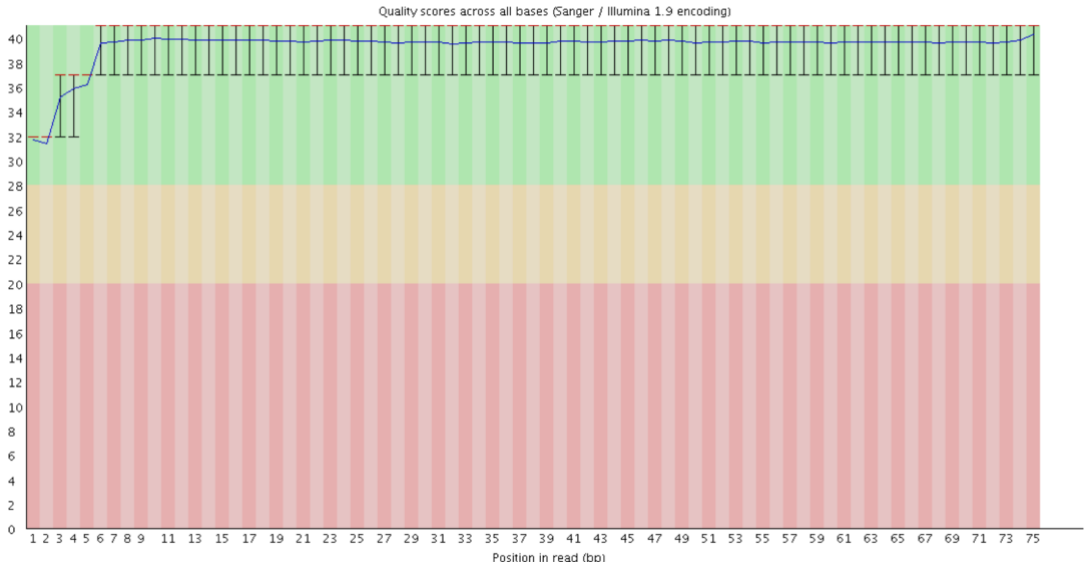

Figure 3 Example of Phred score for one sequenced sample

Future work

As no reference genome are available for *H. panicea*, trimmed reads will be used to design a *de novo* genome using to Trinity (Grabherr *et al.* 2011). Mapping to the newly generated genome will then be undertaken using STAR (Dobin *et al.* 2013) and SAMtools (Li *et al.* 2009). Finally, differential expression analysis will be performed with R studio (R core team 2015) using the package edgeR (Robinson *et al.* 2010).

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