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Echinoderms and Oil Pollution: A Potential Stress Assay Using Bacterial Symbionts

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Oil pollution is a problem in the North Sea. Important sources of oil pollution are spills and drill cutting. Echinoderms are a major component of the macrobenthos in the North Sea (and elsewhere). They tend to be very sensitive to various types of marine pollution. Many species of echinoderms contain symbiotic sub-cuticular bacteria (SCB). The response of *Amphiura filiformis*, *A. chiajei* and *Ophiothrix fragilis*, all of which contain SCB, to oil pollution was studied in laboratory experiments, mesocosms and in the field. Sublethal stress was monitored by examining changes in the tissue loading of SCB. When subjected to hydrocarbon insult, there was a decline in the number of SCB. The potential use of SCB abundance to detect sublethal stress is discussed.

Oil Production Operations in the North Sea

The sources of oil input to the North Sea are numerous and include atmospheric deposition, rivers/land run-off and the dumping of sewage and dredged spoils. A major input arises from oil production activities (NSTF, 1993). Oil exploration and production, though peaking in the late 1970s, is still very active in the North Sea, particularly in its Northern sector. Drill cuttings are a waste product of operations and mainly consist of sediments brought up by the drill. Drilling muds used to lubricate the drill are recycled, but not all of the mud can be removed from the drill cuttings. In the UK sector of the North Sea, the processed drill cuttings are dumped onto the sea bed beneath the oil platform. Despite the development of low-toxicity drilling muds, oil-based muds are still frequently used because of their superior lubrication (GESAMP, 1993). In 1993, 133 wells were using oil-based muds; within the East Shetland basin alone, the input of oil from dumped cuttings amounts to 4000 t (DTI, 1994). Although less than 1% of the North Sea is affected by oil production, drilling operations have had significant local effects (GESAMP, 1990). In addition to this chronic oil contamination of the benthos, there is significant, acute input from major oil spills caused by blowouts and tanker accidents. In 1993 the oil tanker MV *Braer* grounded on the south coast of Shetland, discharging 85 000 t of Gullfaks crude oil into the North Sea.

Traditionally the methods employed to study the impact of oil have concentrated on detecting changes within the benthic community structure, such as diversity indices and the presence or absence of indicator species. These methods are restricted to measuring the lethal effects of pollution. There is increasing interest in the sublethal effects of pollutants on marine organisms, as sublethal stress has important repercussions, not only on the fitness of individual organisms but on the success of the population as a whole (McIntyre, 1984; Bayne *et al.*, 1988). Assays for biochemical and physiological biomarkers are being developed to detect sub-organismal effects of pollutants. Some biomarkers are being included in monitoring programmes, such as EROD induction (an enzyme in the Cytochrome P_{450} detoxification system) in the flatfish, dab (*Limanda limanda* (L)), and the integrity of lysosomes in dab and mussels (*Mytilus edulis* L). However, the current methods all have limitations: flatfish can be very mobile and so their condition may not reflect the immediate environment in which they were caught, while mussels, although sessile, only attach to hard substrates and are restricted to shallow waters. There is a need to develop additional bioassays to complement and, if possible, to improve upon the existing assays.

This paper discusses the effects of oil pollution on the loading of symbiotic sub-cuticular bacteria (SCB) in echinoderms and their possible role in developing a new method for detecting sublethal stress that overcomes some of the problems found in other assays.

Echinoderm Symbionts

Associations between marine invertebrates and bacteria are widespread (Prieur, 1991; Saffo, 1992). Echinoderms often have substantial populations of symbiotic bacteria that are present in the space between the surface coats (cuticle) and the epidermis, hence their being termed sub-cuticular (Fig. 1). SCB have been found in all extant classes of echinoderms (Holland & Nealson, 1978; Féral, 1980). In a survey of SCB distribution in species of echinoderms from the British

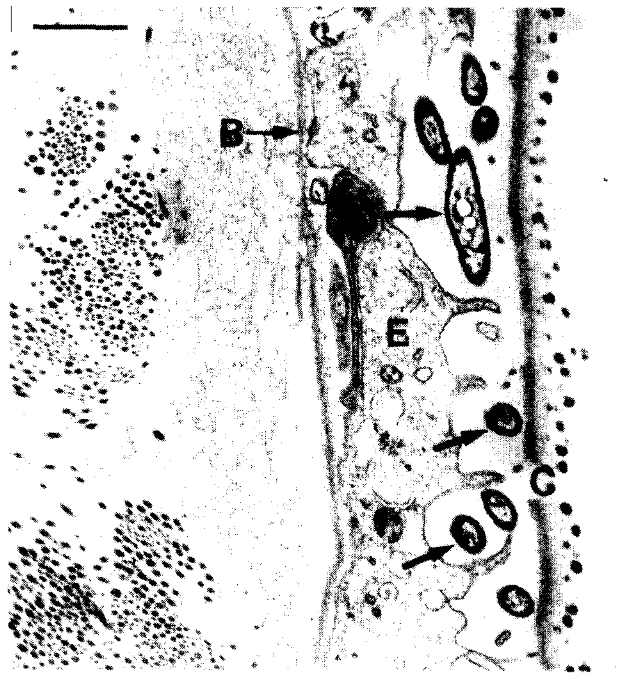

Fig. 1 Transmission electron micrograph of the integument of *Amphiura filiformis*. Note the abundant SCB in the subcuticular space (arrowed); basal lamina (B), cuticle (C), epidermis (E), scale bar = 1 µm.

Isles, over 60% of the 63 species examined contained SCB (Kelly & McKenzie, in press). At present, little is known about the biology of the SCB and the relationship between the echinoderm host and the SCB. Host epidermal cells have been observed phagocytozing SCB. This may be the main mechanism by which the host controls the SCB load, preventing the bacteria becoming invasive while maintaining an optimal SCB population. During preliminary studies, it was noticed that acute stress perturbed the association between the host and the bacteria, leading to a reduced SCB load. To evaluate the possible use of this as an assay for stress, studies were undertaken to examine the response of SCB in brittlestars to drill cuttings and the *Braer* oil spill.

Methods and Results

The SCB load was determined by direct counting using the epifluorescent microscopy method of Hobbie *et al.* (1977) as adapted by Kelly & McKenzie (1992). For making direct counts of bacterial numbers, a piece of each arm was removed from each animal, added to those from other animals, then homogenized using an 'Omni' mechanical tissue homogenizer. The tissue was homogenized at a ratio of 1 g tissue to 2 ml of filtered (0.2 µm) seawater and then mixed with an equal volume of acridine orange. An exact volume of the homogenate (5 µl) was slide-mounted using No. 1 22 × 22 mm coverslips. To estimate the number of bacteria in each homogenate, all the bacteria observed within an eyepiece-mounted Whipple grid (at × 1000 magnification) were counted from 20 randomly selected areas. External or contaminant bacteria were only rarely seen. Obvious differences in size and appearance meant they were readily distinguished from the SCB. The number of bacteria was expressed per gramme of tissue wet weight. For wet weights, tissue samples were first rinsed in filtered (0.2 µm) seawater, shaken to remove excess surface water and then weighed. Microscopical observations and counts of the bacteria were made using a Zeiss Axioscope fluorescent microscope (Filter set 9).

Drill cuttings

The ophiuroids *Ophiothrix fragilis* (Abildgaard) and *Amphiura chiajei* (Forbes) were collected by dredging from Oban Bay on the west coast of Scotland. *Ophiothrix fragilis* was exposed to three concentrations of oil-based drill cuttings: 1/10, 1/100 and 1/1000 dilutions of cuttings (approximately 30 000, 3000 and 300 ppm oil, respectively). Over a 2-week period, the cuttings were replaced daily to maintain the hydrocarbon level and the SCB load monitored. The three treatments appeared to depress the number of SCB (Fig. 2). Within 2 days the SCB load in the heavily treated tanks fell to approximately 50% of the control. Following this decline in SCB, the brittlestars began dying. After 6 days the SCB load in the 1/100 dilution appeared to show some recovery. The reason for this is unknown but it could be an artefact of the low numbers of brittlestars surviving. However, after 6 days all the brittlestars in the heavily treated tanks had died. The brittlestars exposed to the 1/1000 dilution showed a gradual and steady decline in SCB numbers and only one mortality throughout the experiment.

The infaunal brittlestar *A. chiajei* was exposed to a single insult of the drill cuttings at a concentration of approximately 300 ppm. Throughout the experiment there was considerable variation in the SCB load in the control tanks. This meant that it was not possible to show a significant effect of the cuttings, but there was a general depression in the SCB from the treated tanks. The SCB load in the treated brittlestars began to decline to 88% of the controls within 3 days. The decline in SCB continued at a steady rate until day 10 (SCB 54% of control) then seemed to stabilize for the remainder of the experiment (SCB 57% of control).

These preliminary findings suggest that echinoderms were perturbed by drill cuttings. At low concentrations (<300 ppm total hydrocarbons) there was a gradual

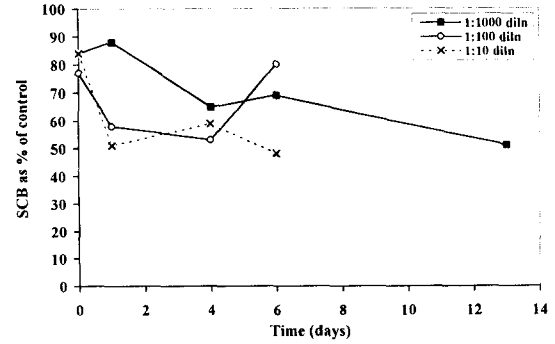

Fig. 2 The effect of three concentrations of drill cuttings in the SCB load of *Ophiothrix fragilis*. SCB load is represented as a percentage of the control. After day 6 all the brittlestars in the 1/10 and 1/100 dilutions had died.

decline in SCB indicating sublethal stress. At stronger concentrations there was a more rapid loss of SCB followed by death. The depression in the SCB population following exposure to cuttings was also found during mesocosm studies on the long-term effects of cuttings on *Amphiura* (unpublished results). In these studies the SCB load gradually declined in the treated tanks until 40 days after the addition of the cuttings. During the following 3 months the SCB levels gradually recovered, presumably as the oil was gradually washed out and degraded.

The Braer *oil spill*

Amphiura filiformis (O.F.Muller), *O. fragilis* and *O. ophiura* (Linnaeus) were collected from sampling sites throughout the Northern North Sea in June 1993 to study the impact of the *Braer* oil spill. At each site the SCB load was determined and compared to the level of oil contamination in the sediment. All three species showed the same trend: a decrease in SCB with increasing contamination of the sediment (Fig. 3). Only for *A. filiformis* were enough samples taken to test this trend statistically and it was found to be significant (Spearman's rank correlation coefficient, $p < 0.05$). Therefore, it is possible that the oil from the *Braer* had sublethal effects on echinoderms as indicated by the decline in SCB.

SCB: A Potential Biomarker?

Echinoderms exposed to hydrocarbons, either from drill cuttings or oil spilled from the *Braer*, were found to have depressed loadings of SCB. The duration and extent of the decline in SCB load was related to the nature of hydrocarbon exposure. A gradual, continual decline was observed with repeated exposure, whereas a transient decrease in SCB was observed following a single insult. Changes in SCB abundance, either from a direct effect on the SCB, indirect effects via the host or by a combination of effects, may be an indication of sublethal stress to the host. Furthermore, a reduction in the SCB load may even be a cause of host stress.

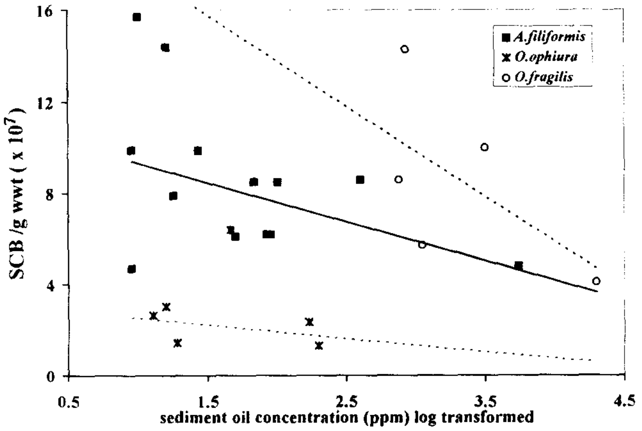

Fig. 3 The relationship between SCB load and the level of oil contamination in *Amphiura filiformis*, *Ophiothrix ophiura* and *O. fragilis*. The SCB load is represented as bacteria numbers per gramme wet weight, and the trend lines for each species are shown.

Assessing the impact of pollutants on marine benthic communities requires a suite of techniques. These should include methods for detecting general stress (such as scope-for-growth) as well as assays to monitor a specific insult, an example being metallothionein production in response to exposure to heavy metals. As noted by Widdows (1978), the response of an animal is never in terms of individual physiological rates but rather as a whole organism. This can only be assessed by integrating the various individual physiological and biochemical assays, thereby producing an overall assessment of the impact on the ecological fitness of an individual and, from them, the population. The recognition of the importance of detecting sublethal effects has led to many bioassays being suggested and promoted as biomarkers. However, common weaknesses include low ecological relevance, lack of consistent response, species specificity, seasonal variability, low level of precision, slow response time and requirements for expensive/complex equipment, and many are also unsuitable for use in the field (Abel & Axiak, 1991; Forbes & Forbes, 1994).

In contrast, the response of echinoderms to pollutants is of great ecological relevance, since echinoderms are geographically widespread, occur in most marine habitats and are often the dominant species in benthic macrofaunal communities in both abundance and biomass (Brun, 1969; Bowmer *et al.*, 1986). Some of the classic benthic communities are named after the echinoderms found within them. The SCB assay is based upon a symbiotic relationship. It is possible for the stressor to be having an indirect effect via the host, a direct effect on the SCB or possibly a mixture of both. Since the stressor could be acting directly or indirectly (via the host) on the SCB, perhaps SCB load is sensitive to many forms of stress. A decline in SCB has been noted not only to hydrocarbon exposure but also to thermal shock and nutrient limitation (unpublished results). This may indicate that it is a general response to stress and not specific to a particular pollutant. The assay may be regarded as a bacterial assay with the echinoderm acting as a carrier. Thus, subtle effects of the stressor may be detected earlier, due to the more rapid regeneration rates of bacteria than in other test organisms. The quantification of SCB is relatively simple to perform and inexpensive.

One important advantage of the assay is that it can be applied to all levels of monitoring—for laboratory-based experiments, mesocosms and for examining effects in the field. Echinoderms are easy to collect and maintain under laboratory conditions. The effects of potentially toxic compounds or contaminated sediments could be readily tested under laboratory-controlled conditions. Due to the complexity of ecosystems it is difficult to predict the impact of pollutants in the field (Abel & Axiak, 1991). Echinoderms have been successfully used to demonstrate the effects of pollutants under semi-natural conditions (mesocosms) and in the field. There was no evidence of a marked seasonal effect on SCB load in *A. chiajei* and *O. fragilis* from Oban Bay (unpublished results). An important attribute for field monitoring is that, although echinoderms are motile, many species are sedentary. Therefore, the effects observed on SCB load can be related directly to the conditions at that particular location. As mentioned, echinoderms are common around the British Isles, making it possible to follow and sample along pollution gradients from a point source.

In summary, there are promising indications that changes in SCB load could form a novel assay for sublethal stress. It would be particularly useful if used in tandem with other indicators of host stress such as MFO induction and scope-for-growth. Work is continuing on these aspects and on refining knowledge of the effects of pollutants on the SCB, particularly the establishment of dose–response relationships. It would be useful to develop improved methods of determining SCB load. Although direct counting is accurate, it is slow. The use of fluorescent labelled antibody or rRNA targeted probes to measure the SCB load may be the best approach to refining the quantification method.

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