SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 24, No. 5, pp. 1219–1227, 2005
© 2005 SETAC
Printed in the USA
0730-7268/05 $12.00 + .00

COMPARATIVE TOXICITY OF TWO OIL DISPERSANTS, SUPERDISPERSANT-25 AND COREXIT 9527, TO A RANGE OF COASTAL SPECIES

Alan Scarlett,† Tamara S. Galloway,*‡ Martin Canty,‡ Emma L. Smith,† Johanna Nilsson,‡ and Steven J. Rowland†

†School of Earth, Ocean, and Environmental Sciences, and ‡School of Biological Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA United Kingdom

(*Received* 2 *July* 2004; *Accepted* 5 *November* 2004)

Abstract—The acute toxicity of the oil dispersant Corexit 9527 reported in the literature is highly variable. No peer-reviewed data exist for Superdispersant-25 (SD-25). This study compares the toxicity of the two dispersants to a range of marine species representing different phyla occupying a wide range of niches: The marine sediment-dwelling amphipod *Corophium volutator* (Pallas), the common mussel *Mytilus edulis* (L.), the symbiotic snakelocks anemone *Anemonia viridis* (Forskål), and the seagrass *Zostera marina* (L.). Organisms were exposed to static dispersant concentrations for 48-h and median lethal concentration (LC50), median effect concentration (EC50), and lowest-observable-effect concentration (LOEC) values obtained. The sublethal effects of 48-h exposures and the ability of species to recover for up to 72 h after exposure were quantified relative to the 48-h endpoints. Results indicated that the anemone lethality test was the most sensitive with LOECs of 20 ppm followed by mussel feeding rate, seagrass photosynthetic index and amphipod lethality, with mussel lethality being the least sensitive with LOECs of 250 ppm for both dispersants. The results were consistent with current theory that dispersants act physically and irreversibly on the respiratory organs and reversibly, depending on exposure time, on the nervous system. Superdispersant-25 was found overall to be less toxic than Corexit 9527 and its sublethal effects more likely to be reversible following short-term exposure.

Keywords—Dispersants *Anemonia viridis* *Corophium volutator* *Mytilus edulis* *Zostera marina*

INTRODUCTION

Faced with the prospect of an oil spill coming ashore or passing over reefs, decisions have to be made swiftly as to how best to deal with the situation. One option is to use chemical dispersants to break up the slick into a large number of small droplets. Once broken up, the slick poses less of a physical risk to seabirds or marine mammals but may transfer oil into the water column and possibly to the benthos. Within estuaries, inlets, enclosed bays, or shallow water reefs, the concentration of the dispersants alone may be sufficient to cause toxic effects. In the United Kingdom, dispersants cannot be used in water less than 20 m deep or within one nautical mile of such [1] without the permission of the Department for Environment Food and Rural Affairs; similar rules relating to sensitive habitats such as coral reefs and mangroves exist in tropical regions [2]. Hence, the option to use dispersants within estuaries, inlets, and shallow water does exist and it is in such circumstances that difficult decisions on how best to protect the environment and commercial operations have to be made. The handling of large volumes of dispersant under difficult conditions may result in accidental release of potentially toxic chemicals into the sea. Research into the toxicity of dispersants has been reported widely [3,4] and companies continue to improve the efficiency of the chemicals and reduce their toxicity. In the United Kingdom, the oil dispersant Superdispersant-25 (SD-25) is now the Maritime and Coastguard Agency's main stockpiled chemical for spraying onto oil slicks at sea. No data exist within peer-reviewed literature for SD-25. However, SD-25 in association with oil meets all the relevant clauses of Warren Spring Laboratory Specification LR 448(OP) and has been approved as a type-2, as well as a type-3, dispersant under test qualification CSR 4600/8902798 (Oil Slick Dispersants Ltd. product profile, cited May 5, 2004; http://www.croftpark.co.uk/osd-products.html). Superdispersant-25 has been tested by the Center for Environment, Fisheries, and Aquaculture Science and has been found to be of low toxicity to *Crangon crangon* (brown shrimp) for use at sea and on beaches, and *Patella vulgata* (common limpet) for use on rocky shores; it is licensed under the Ministry of Agriculture, Fisheries, and Food, Food and Environment Protection Act 53/98 (Oil Slick Dispersants Ltd. product profile, cited May 5, 2004; www.croftpark.co.uk/osd-products.html). Corexit 9527 has been tested extensively in the laboratory and used on oil spills since 1978 [4]. A considerable number of toxicity reports exist concerning a wide variety of species, reviewed by George-Ares and Clark [3]. Thus Corexit 9527 provides a useful comparative toxicant for the study of SD-25.

The use of dispersants within enclosed bodies of water may pose a threat to a diverse range of species. This study compares the toxicity of SD-25 with that of Corexit 9527 to the marine sediment-dwelling amphipod *Corophium volutator* (Pallas), the blue mussel *Mytilus edulis* (L), the symbiotic snakelocks anemone *Anemonia viridis* (Forskål), and the seagrass *Zostera marina* (L.). The mudshrimp *C. volutator* is distributed widely around the coasts of western Europe and northeast America, and is significant in structuring and sustaining the ecology of near-shore sediment communities [5,6]. *Corophium volutator* is now used commonly as a European acute toxicity test organism [7–12]. Amphipods occupying a similar niche exist in other regions, e.g., *Ampelisca abdita* (Mills) also are used for toxicity testing. Blue mussels mainly occur on exposed rocky

* To whom correspondence may be addressed (tamara.galloway@plymouth.ac.uk).

shores and are distributed widely from the Arctic to the Mediterranean with related species distributed worldwide. Mussels have been used for long-term monitoring projects such as the Global Mussel Watch [13], field surveys [14], and Scope for Growth studies [15], allowing a large body of knowledge to accumulate regarding their acute and sublethal responses to stressors. Snakelocks anemones occur on intertidal rocky shores and are associated closely with seagrass beds [16]. As symbionts possessing zooxanthellae, anemones have been used as surrogate organisms for the study of coral organisms (e.g., [17,18]). Eelgrass, *Z. marina*, is a marine angiosperm with a worldwide distribution and is protected strictly under the Berne Convention [19]. North Atlantic populations have suffered great losses during the last century [20] and, as a consequence, *Z. marina* is now deemed to be scarce. Eelgrass plants grow well under laboratory conditions and have proved to be a robust test species if chlorophyll fast fluorescence transient is used to measure plant health [21]. Taken together, these species represent a broad range of organisms that might be affected by dispersant use. Some degree of extrapolation to other similar species can be justified from the toxicity data obtained.

The aim of this study was to compare the acute and sublethal toxicities of the two dispersants, Superdispersant-25 and Corexit 9527, following 48-h static exposures. The extent of recovery of each species was documented for up to 72 h with regard to changes in the no-observable-effect concentration (NOEC) and LOEC values. Behavioral responses of test animals also were recorded.

MATERIALS AND METHODS

Collection and maintenance of organisms

Corophium volutator and sediment were collected from an intertidal area of the Avon estuary near Aveton Gifford, South Devon, United Kingdom (ordinance survey grid reference: SX 683 467). Amphipods were sieved from the upper 5 cm of sediment and transported back to the laboratory within 1 h, where they were placed in 5-L culture tanks lined with field-collected, sieved (<300-μm) sediment. The tanks were filled with filtered seawater 25 ± 1 practical salinity units (psu), which was aerated and maintained at 15 ± 1°C with a 12:12-h light:dark cycle. The animals were fed weekly with five drops standard aquarium invertebrate food (Waterlife Invert Food, Waterlife Research Industries, Longford, UK; Liquifry Marine, Interpret Ltd., Dorking, UK; Roti-Rich, Florida Aqua Farms, Dade City, FL, USA; and dried algae) and the water was replaced 24 h after feeding. Amphipods were maintained under the above conditions for one to two weeks after removal from the field to acclimate them to experimental conditions.

Mussels were collected from Port Quin, on the North Cornwall coast, United Kingdom (ordinance survey grid reference: SW 972 905). Organisms of 30-mm ± 5 mm length were removed carefully from the rock by cutting the byssal threads and transported back to the laboratory within 2 h. Any epifauna and epiphytes were removed from the mussels, which then were placed in 20-L tanks filled with filtered 34 ± 1 psu seawater. Other conditions were as above.

Anemones were collected from Jennycliff Bay, Plymouth Sound on the South Devon coast, United Kingdom (ordinance survey grid reference: SX 491 523) and transported back to the laboratory within 1 h. Maintenance conditions were same as for the mussels.

Eelgrass was collected from the Yealm estuary, South Devon, United Kingdom (ordinance survey grid reference: SX 530 476) and transported to the laboratory within 1 h. The outer leaves were removed so that the three youngest leaves remained. These leaves were wiped with paper to remove epiphytes and shortened to a maximum length of 300 mm above the sheath. Roots were standardized to three rhizome segments. The plants were placed in 20-L tanks filled with filtered 34 ± 1 psu seawater without any sediment or additional nutrient. Other conditions were as above.

Preparation of test solutions

Corexit 9527 was supplied gratis from the U.S. Minerals Management Service and SD-25 was obtained from the U.K. Maritime and Coastguard Agency. Nominal exposure concentrations were prepared by direct syringe injection or pipetting of dispersant into seawater adjusted to the required salinity for the toxicity tests as appropriate (25 ± 1 psu for *Corophium*, 34 ± 1 psu for other spp.) and vortex mixing for 3 min at high speed.

Rationale and general test conditions

Toxicity tests of dispersants have been performed using short-term, static, continuous flow-through and spiked declining flow-through exposures. Exposure conditions have a considerable influence on the reported toxicity values with spiked declining exposures giving much higher LC50 values, approximately 3 to 23 times greater, than continuous exposures [3]. Here we used static exposures of 48-h duration noting that the length of exposure does not necessarily produce an incipient LC50 for any of the species tested during the 48-h exposure and longer exposure, therefore, may result in lower LC50 values. As well as acute toxicity, the ability of organisms to recover in clean seawater for up to 72 h was assessed relative to the 48-h endpoints. The species-specific sublethal endpoints for each test are described in the following paragraphs.

Test vessels (2-L Pyrex beakers) were maintained at 15 ± 1°C with a 12:12 h light:dark cycle. Beakers were sealed loosely with Parafilm® M (Pechiney Plastic Packaging, Menasha, WI, USA) and aerated via a glass Pasteur pipette. Dissolved oxygen, pH, temperature, and salinity were measured after 0, 24, and 48 h in one replicate from each treatment. Dissolved oxygen was measured in all replicates at the beginning and end of the experiment. Three replicate vessels per treatment were used in all tests except the mussel feeding-rate bioassay in which nine replicate vessels per treatment were used. Test results were analyzed statistically using one-way analysis of variance after checking for normality and homogeneity of variances; 48-h LC50 values were derived using the trimmed Spearman-Kärber method. The EC50 values were obtained from regression of log-transformed data.

Corophium volutator

In order to obtain a concentration-response relationship, nominal exposure concentrations of 0, 50, 125, 175, 213, 250, 375, and 500 ppm Corexit 9527 and SD-25 were prepared as above. Sieved sediment (<300 μm), approximately 160 ml, was placed in the test vessels to a depth of 15 mm and 1 L of test solution added and allowed to settle for 2 h before 20 amphipods (size range 3–7 mm) were introduced via a plastic Pasteur pipette to each test vessel, a total of 60 amphipods per treatment. Beakers were inspected for amphipod activity and behavior after 18, 24, and 42 h. At the end of the exposure period of 48 h, the sediment was sieved (300 μm) and the number of live, dead, and missing individuals recorded. Miss-

ing individuals were assumed to have died. Surviving amphipods were placed in clean sediment and seawater for a 24-h recovery period to assess their ability to burrow.

Mytilus edulis

Nominal exposure concentrations of 0, 80, 130, 200, 250, and 320 ppm Corexit 9527 and SD-25 were prepared as above. Three mussels, length 30 mm ± 5 mm, were placed in the test vessels with 1 L of test solution, a total of nine mussels per treatment. At the end of the exposure period, the mussels were categorized as: Closed, open but able to close when stimulated, or open but failed to close when stimulated. Mussels in the former two categories were placed in clean seawater for a further 72 h. Open mussels that failed to respond to stimuli were considered dead and were excluded from the recovery trial. Following the recovery period of 72 h, mussels were reassessed. An additional test was performed with a single toxicant treatment of 50 ppm for both dispersants plus controls using only one mussel per test vessel but with nine replicates per treatment. At the end of the 48-h exposure period, the feeding rate of the mussels was assessed based on methodology described by Donkin et al. [22]. Mussels were placed individually in 400-ml glass beakers containing 350 ml of clean seawater. After a 10-min acclimation period with slow vortex mixing, 500 μl of *Isochrysis* sp. algal solution was added to give approximately 2×10^4 cells ml^{-1}. A 20-ml water sample was removed immediately from all the beakers upon the addition of the algae and retained in vials for algae enumeration. Further samples were taken after 15 and 30 min. Algal particles were analyzed using a Beckman Z2 Coulter particle count and size analyzer (Beckman Coulter, Wycombe, UK), which was set to count particles between 3 to 10.43 μm. From the loss of algal particles during the 30-min period, the feeding rates of the mussels were determined.

Anemonia viridis

Scoping tests had indicated that anemones were highly sensitive to both dispersants; therefore, a lower concentration range of 0, 10, 20, 40, 80, and 160 ppm was used followed by a 24-h recovery period. One anemone per test vessel was exposed to 1 L of test solution, a total of three anemones per treatment. At the end of the 48-h exposure period, the anemones were classified as having extended or retracted tentacles. The tentacles were stimulated gently with a glass rod and their response recorded. Anemones that were insensitive to stimuli were classed as moribund because they would be unable to feed or defend themselves from predation and, therefore, were unlikely to survive in their natural environment. The anemones then were placed in clean seawater for a 24-h recovery period and reassessed. A confirmatory test was performed using three anemones per test vessel at 20 and 30 ppm, a total of nine anemones per treatment. The recovery period was extended to 72 h to test if further recovery was possible. The effect of the dispersants on the density of zooxanthallae within the tentacles of *A. viridis* also was assessed using anemones exposed to the 0 to 160 ppm concentration range following 48-h exposure and 24-h recovery. Zooxanthallae were separated from the host material after homogenization of weighed tentacles in a Wheaton glass homogenizer in 1 ml of buffer solution (50 nM HEPES, 1 nM ethylenediaminetetraacetate, pH 7.4). Whole homogenate was centrifuged for 3 min at 735 G (Eppendorf 5415C, Eppendorf UK, Cambridge, UK). The supernatant was discarded and the insoluble plant material retained and resuspended in buffer. Centrifugation was repeated until samples were free of host tissue. The resulting pellet was resuspended in buffer to give a cell density of about 1×10^6 cells ml^{-1}. Zooxanthallae numbers were determined by replicate counts ($n = 5$) and converted to zooxanthallae per wet weight of tentacle.

Zostera marina

Because mortality is not a practical parameter for a plant toxicity test, the photosynthetic efficiency of eelgrass was examined using the chlorophyll fast fluorescence JIP transient (from 50 μs to 1 s; J is a characteristic step at 2 ms, I is an intermediate step at 30 ms, and P is maximum fluorescence) measurements [23,24]. Three plants per exposure vessel were exposed to a concentration range of 0, 80, 130, 200, 320, and 500 ppm dispersant, a total of nine plants per treatment, followed by a 24-h recovery period in clean seawater. Plants were dark-adapted for 5 min using a clip system before illumination with 660 nm light from a light-emitting diode source built into the fluorometer sensor. Fluorescence measurements at F_0 (50 μs), $F_{300\mu s}$ F_j (2 ms), and F_m (tF_{max}) were recorded using a Handy PEA plant efficiency analyzer (Hansatech Instruments, Kings Lynn, Norfolk, UK) after 24 and 48 h. The Biolyzer© software (Ronald Maldonado-Rodriguez, Bioenergetics Laboratory, University of Geneva, Geneva, Switzerland, 2002) was used to load the full fluorescence transients and to calculate the JIP parameters according to the equations of the JIP-test [23,24]. The effect of the dispersants on the plant's photosynthetic apparatus was assessed by statistical comparison of the main parameters: Yield of primary photochemistry expressed in ratio of variable to maximal fluorescence (Fv/Fm), performance index (PI), and the area between the fluorescence curve and the maximal fluorescence intensity. Ten measurements per exposure vessel were taken at random points on the leaves avoiding any necrotic lesions (minimum of 10 mm distance). The plants then were placed in clean seawater and further measurements recorded after 24 h.

RESULTS

Physical conditions

Dissolved oxygen was above 70% saturation, pH in the range 7.8 to 8.2, salinity 25 ± 1 psu (*Corophium* test) or 34 ± 1 psu (other spp. tests), and temperature 15 ± 1°C during the exposure period in all test vessels.

Corophium volutator

No activity was observed after 18 h at nominal dispersant concentrations at or below 125 ppm, or within the controls. At dispersant concentrations of 175 ppm and above, individuals were showing signs of stress: Either crawling on the sediment surface or swimming erratically. The activity caused greater turbidity within treatments compared to the controls. After 24 h, several moribund individuals were visible in exposure treatments of 375 and 500 ppm for both Corexit 9527 and Superdispersant-25. After 42 h of exposure, moribund individuals also were visible at and above 175 ppm for both dispersants, although this clearly was greater within the Corexit 9527 treatments. By the end of the experiment, little activity was observed, resulting in a reduction in turbidity, especially at the higher concentrations.

Mortality was less than 1% within the controls and 100% at the highest nominal concentration of 500 ppm for both Corexit 9527 and SD-25 (Fig. 1). Although there was a sharp

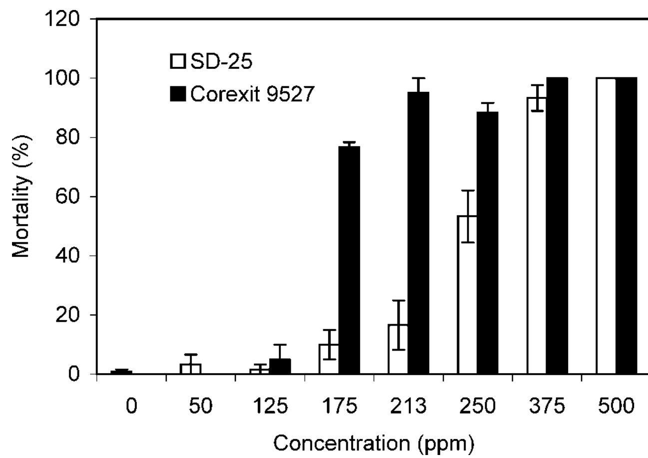

Fig. 1. Mortality (mean ± standard error) of the mudshrimp *Corophium volutator* following a 48-h static exposure to the oil dispersants Corexit 9527 and Superdispersant-25 (each treatment contained three replicates of 20 amphipods).

fall in survival of *C. volutator* exposed to 175 ppm Corexit 9527, a more gradual decline was observed within the SD-25 treatments. The LC50 (48-h) values of 159 (95% confidence limits 145–173 ppm) and 260 ppm (95% confidence limits 240–282 ppm) were calculated for Corexit 9527 and SD-25, respectively (Table 1). The NOEC was 125 ppm for both dispersants. Mortality of Corexit 9527 exposed amphipods was significantly ($p < 0.05$) greater than those exposed to SD-25. All of the surviving individuals exposed to SD-25 concentrations up to 175 ppm were able to swim normally and were able to rebury in clean sediment, although swimming activity still was observed 3 h following introduction into the clean seawater; all survived the recovery period. Survivors from Corexit 9527 also were able to rebury and survive the recovery period with exposures up to 125 ppm. However, above 175 ppm, 100% of amphipods failed to recover from Corexit 9527.

Mytilus edulis

Control mussels appeared active and healthy (i.e., open and responsive to stimuli) throughout the test period but all dispersant-exposed organisms were classified as either closed or dead at the end of the 72-h recovery period (Table 2). Assessment of mortality after 48 h was problematic because some mussels that initially appeared dead (i.e., open and failing to

Table 2. Responses of the mussel *Mytilus edulis* after 48-h dispersant exposures (48 h) and a 72-h recovery period (Rec.) Mussels from all concentrations were pooled (Superdispersant-25 [SD-25] $n = 45$ and Corexit 9527 $n = 45$; Control $n = 9$ and classified as closed (closed), open and responsive to stimuli (active), or open and unresponsive to stimuli (dead)

	Closed (%)		Active (%)		Dead (%)	
	48 h	Rec.	48 h	Rec.	48 h	Rec.[a]
Control	0	0	100	100	0	0
SD-25	44	58	36	0	20	42
Corexit 9527	67	51	11	0	22	49

[a] Dead mussels from the 48-h exposure were excluded from the recovery vessels but are recorded in the recovery column.

close promptly when touched) were able to close very slowly and then remain closed. Therefore, only those that absolutely failed to close when touched were deemed to have died (Fig. 2a). At the lowest concentration of 80 ppm, the bivalves exposed to Corexit 9527 experienced about 20% mortality but variation was high and significant mortality ($p < 0.05$) only occurred at 250 ppm for Corexit 9527–and SD-25–exposed organisms (Table 1). High variation within treatments occurred at all treatment levels and reliable LC50 values could not be calculated. Following 72 h in clean seawater, the number of dead mussels had increased, but NOEC and LOEC values were unchanged (Fig. 2b). Sublethal effects were assessed at dispersant concentrations of 50 ppm for 48 h using a feeding rate bioassay ($n = 9$). Two mussels within the Corexit 9527 exposure died and were omitted from the bioassay. Feeding rates were reduced significantly ($p < 0.05$) for both dispersant exposures with SD-25 rates reduced to 9.8% of controls and Corexit 9527–exposed mussels only 2.6% (Fig. 3). Corexit-exposed mussels had feeding rates significantly ($p < 0.05$) less than that of SD-25–exposed organisms.

Anemonia viridis

The anemones were highly sensitive to both dispersants with 55% of SD-25–exposed and 100% of Corexit 9527–exposed organisms insensitive to stimuli at 20 ppm (Fig. 4a). At 40 ppm for both dispersants, all organisms had retracted tentacles that failed to respond to stimuli and were assessed to be moribund. Above 80 ppm, anemone tissue was starting to decompose. The concentration range between no effect and

Table 1. Comparison of toxicity estimates for Superdispersant-25 and Corexit 9527 with four marine species

		Corexit 9527 concn. (ppm)				Superdispersant-25 concn. (ppm)			
Species	Test	NOEC[a]	LOEC[b]	LC50 or EC50[c]	Confidence limits	NOEC	LOEC	LC50 or EC50	Confidence limits
Anemonia viridis	No response to stimuli	10	20	15[d]	NA[e]	10	20	20[a]	NA
Corophium volutator	Mortality	125	175	159	145–173	125	175	260	240–282
Mytilus edulis	Mortality	200	250	—	—	200	250	—	—
M. edulis	Feeding rate	<50	50	NA		<50	50	NA	
Zostera marina	JIP-test[f] PI[g]	<80	80	55	28–150	<80	80	386	339–439

[a] NOEC = no-observed-effect-concentration.
[b] LOEC = lowest-observed-effect-concentration.
[c] LC50 = median lethal concentration; EC50 = median effect concentration.
[d] Interpolated from data only, not derived from model.
[e] NA = not applicable.
[f] JIP-test = measurements acquired from the fast fluorescence transient.
[g] PI = performance index.

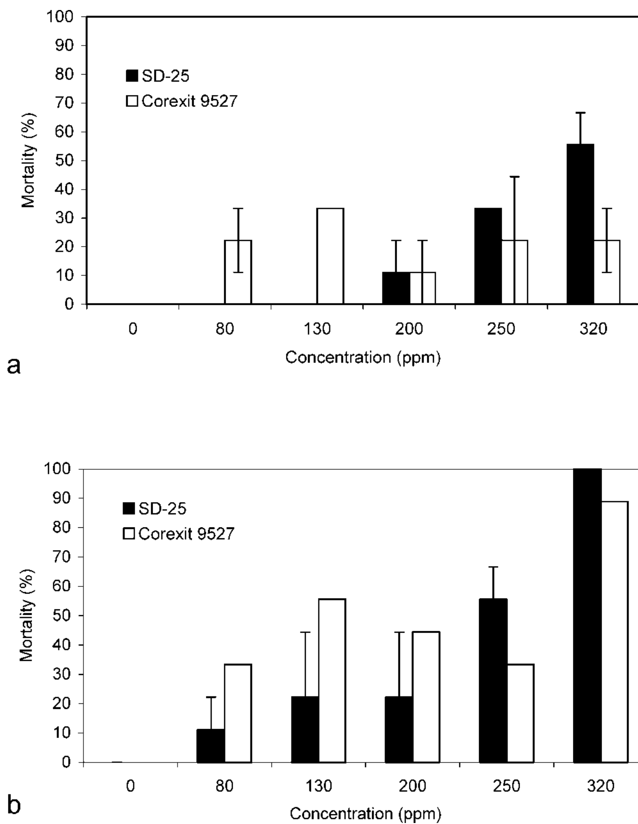

Fig. 2. Mortality (mean ± standard error) of the mussel *Mytilus edulis* following a 48-h static exposure (**a**) and a further 72-h recovery period (**b**) to the oil dispersants Corexit 9527 and Superdispersant-25 (each treatment contained three replicates of three mussels).

100% mortality was too small to calculate LC50 values using the Spearman-Kärber method. However, interpolation of the data indicated EC50 values of about 20 ppm for SD-25 and about 15 ppm for Corexit 9527. The NOEC and LOEC (48-h) values were 10 ppm and 20 ppm, respectively for both dispersants (Table 1). Following recovery in clean seawater,

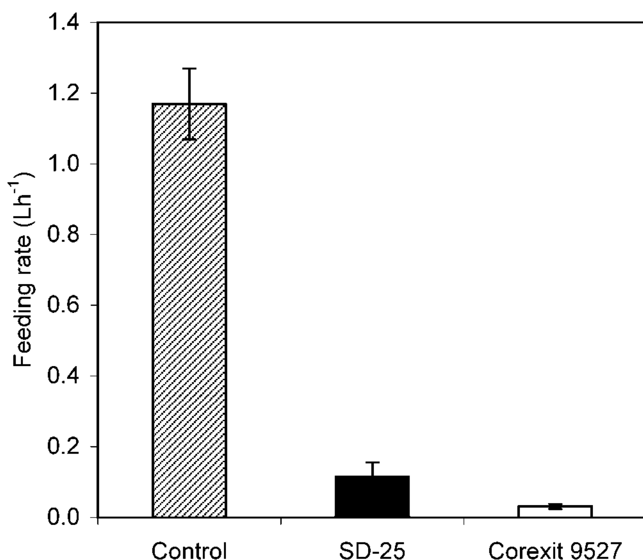

Fig. 3. Feeding rates (mean ± standard error) of the mussel *Mytilus edulis* following a 48-h static exposure of 50 ppm to the oil dispersants Corexit 9527 and Superdispersant-25 (each treatment contained nine replicates of one mussel).

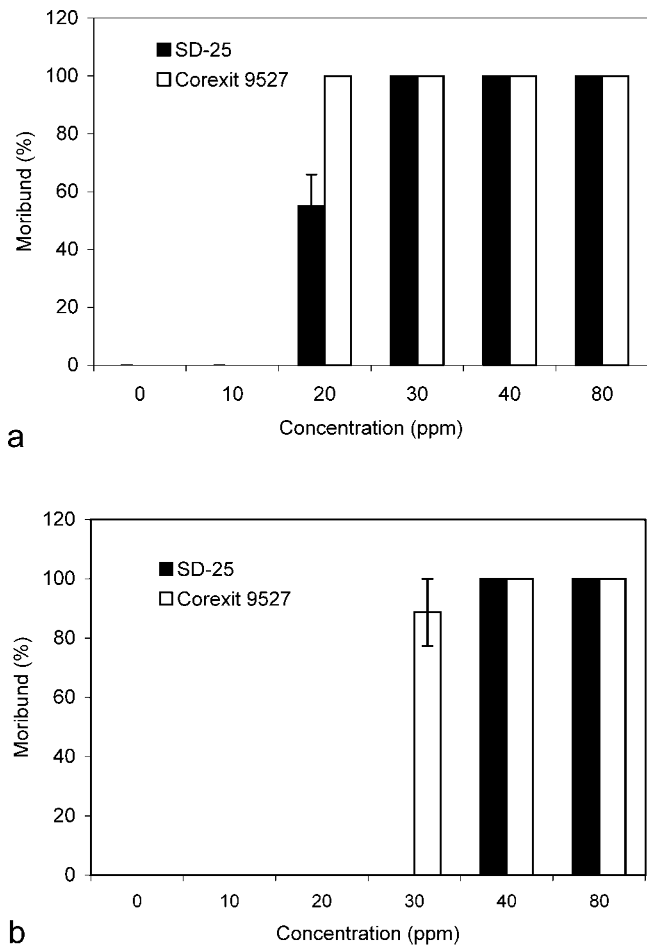

Fig. 4. Percentage of moribund *Anemonia viridis* (mean ± standard error), following a 48-h static exposure (**a**) and a further 24-h recovery period (**b**) to the oil dispersants Corexit 9527 and Superdispersant-25 (the 20- and 30-ppm treatments contained three replicates of three anemones; all other treatments contained three replicates of one anemone).

all of the anemones previously exposed to 30 ppm SD-25 and below were able to respond to stimuli, as did all organisms previously exposed to Corexit 9527 at 20 ppm (Fig. 4b); however, 89% of the anemones previously exposed to 30 ppm Corexit 9527 failed to respond to stimuli (Fig. 4b). No significant differences were observed in zooxanthallae densities.

Zostera marina

All of the main parameters, Fv/Fm, PI, and area, were reduced at the lowest exposure of 80 ppm after 24 and 48 h for both dispersants (Fig. 5); the NOEC for both dispersants was <80 ppm. The PI was the most-sensitive parameter giving 48-h EC50 values of 386 ppm and 55 ppm for SD-25 and Corexit 9527, respectively. At the lowest exposure concentration there was no significant difference between the dispersants; however, at 130 ppm and above, Corexit was significantly ($p < 0.05$) more toxic for all main parameters. Leaves of the Corexit-exposed plants turned brown at 200 ppm and the outer leaves started to become detached, leaving only the more protected inner leaf from which to take measurements. As well as the main parameter values obtained from the PEA, the JIP-test allows calculation of various bio-physical expressions, such as specific fluctuations and yields, and phenomenological fluctuations. To visualize the effect of the dispersants on the

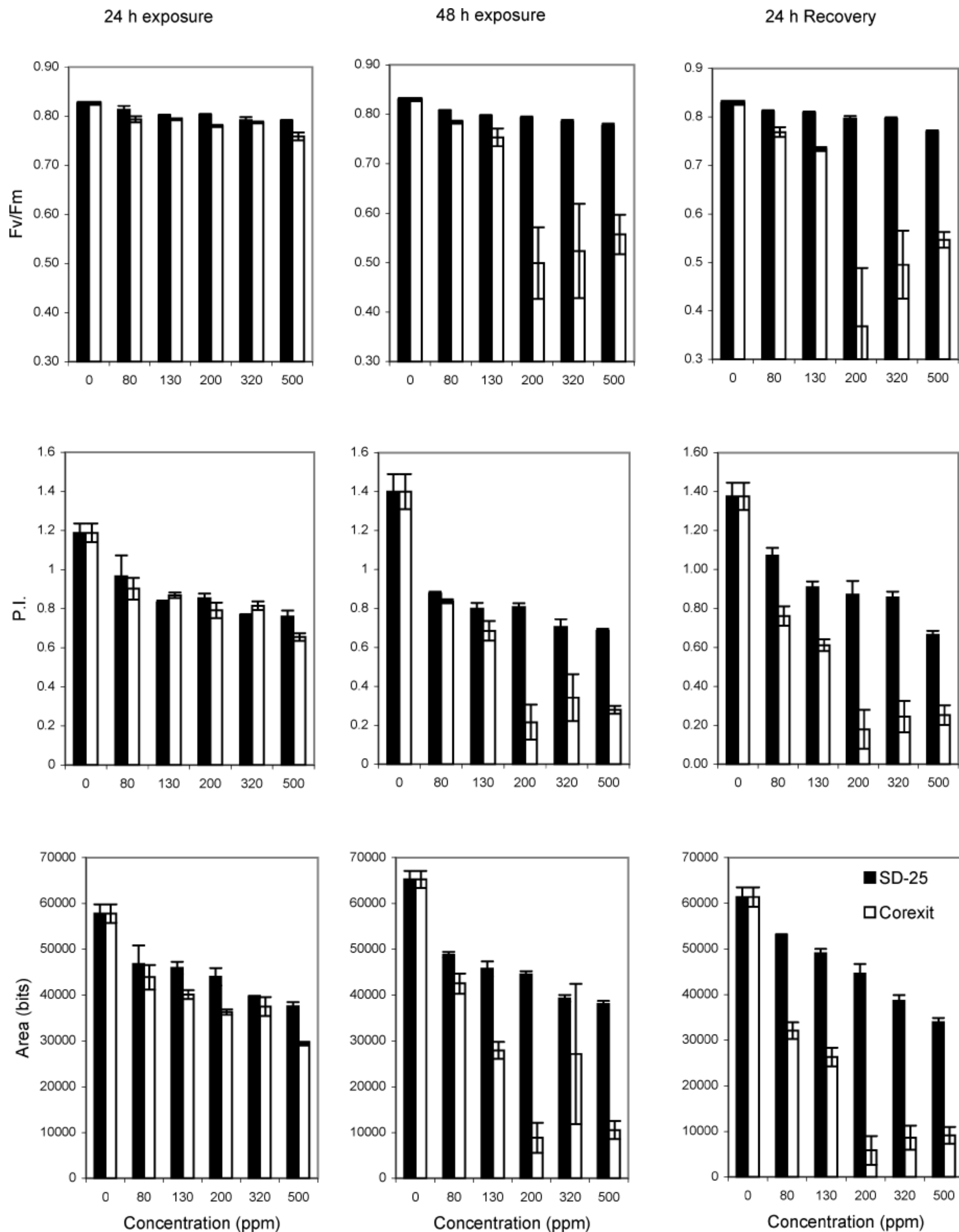

Fig. 5. Photosynthetic parameters (Fv/Fm), performance index (PI), and area (mean ± standard error), measured in the leaves of *Zostera marina* after 24- and 48-h exposure, and 24-h recovery in clean seawater, to the oil dispersants Corexit 9527 and Superdispersant-25 (each treatment contained three replicates of three plants from which 10 measurements were taken).

eelgrass leaves, pipeline models have been calculated and drawn on the basis of experimental signals from the 200-ppm concentrations and compared to control leaves (Fig. 6). The SD-25–exposed plants were able to recover slightly after 24 h in clean seawater, e.g., mean PI values rose from 0.88 (standard error [SE] 0.01) after 48 h at 80 ppm exposure to 1.07 (SE 0.04) following recovery. Corexit-exposed plants failed to recover, e.g., mean PI values were 0.84 (SE 0.01) after the 48-h exposure to 80 ppm and fell to 0.76 (SE 0.07) at the end of the 24-h recovery period (Fig. 4).

DISCUSSION AND CONCLUSION

This is, to our knowledge, the first report of toxicity data for SD-25 that reveals its acute toxicity to a range of marine

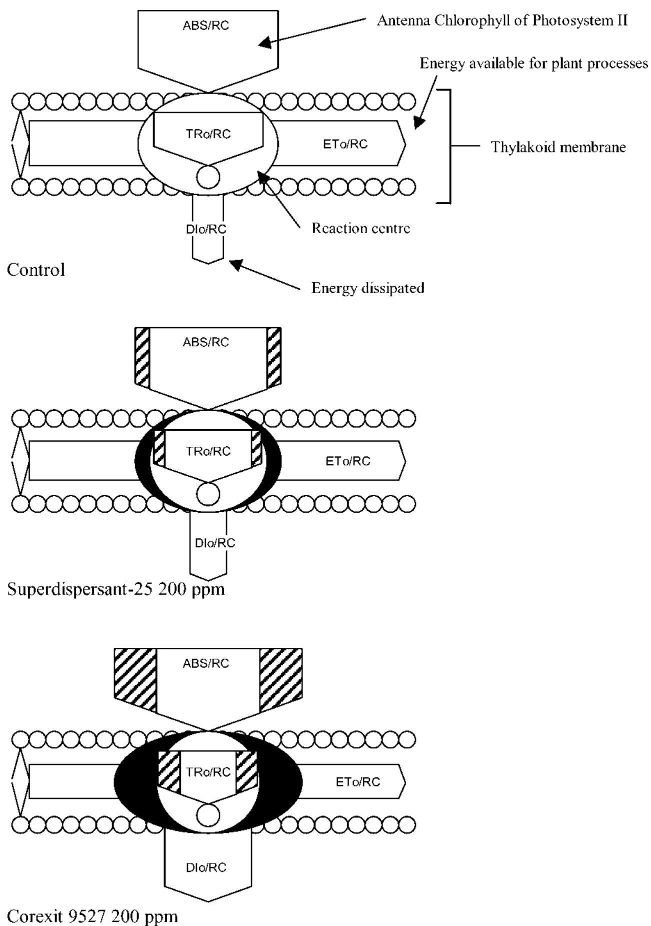

Fig. 6. Comparison of membrane models of the Photosystem II (PSII) apparatus in control plants (top), and 48-h exposure to 200 ppm Superdispersant-25 (middle) and Corexit 9527 (bottom). The models represent the specific activities expressed as fluctuation per reaction center (RC). The relative magnitude of each activity or fluctuation is shown by the width of the corresponding arrow. Absorption flux (ABS) is proportional to the concentration of antenna chlorophyll and the average antenna size is given as ABS/RC. This expresses the total absorption flux of PSII antennae chlorophyll divided by the number of active, in the sense of primary quinone (Q_A)-reducing, reaction centers. The absorption and trapping by PSII units with a heat sink center (non-Q_A–reducing) is indicated as the hatched parts of the arrows ABS/RC and energy flux for trapping (TR_o/RC). The antenna belonging to the PSII units with heat sink centers is drawn in black and the antenna that belongs to the active centers is drawn in white. The degree of stress, thus, is indicated by the reduction in ABS/RC and TR_o/RC leading to a greater dissipation of energy in the form of heat (Dl_o/RC) and a decrease in the useful energy available to the plant (ET_o/RC).

species to be significantly lower ($p < 0.05$), in the majority of tests, or equivalent (mussel mortality and anemone tests) to the widely used dispersant Corexit 9527. Dispersants contain surfactants, which may be nonionic or anionic, dissolved, or suspended in solvents. Corexit 9527 is a mixture of both nonionic (48%) and anionic (35%) surfactants in an aqueous solvent containing ethylene glycol monobutyl ether (17%); the surfactants include ethoxylated sorbitan mono- and trioleates (nonionic) and sodium dioctyl sulfoccinate (anionic) [25], but little is known about the constituents of SD-25 except that it is a blend of glycol and glycol ether solvents, combined with nonionic and anionic surfactants [26]. Dispersants are thought to act physically and irreversibly on the respiratory organs and reversibly, depending on exposure time, on the nervous system [27]. Surfactants can bind to and disrupt cellular phospholipid bilayers altering the transmembrane sodium gradient [28].

A review of Corexit 9527 toxicity data by George-Ares and Clark [3] covering 28 reports and 37 aquatic species, found no apparent trend in the reported sensitivity between taxa. Toxicity (24–96–h LC50 or EC50) ranged from 1.6 to >1,000 ppm. Amphipod LC50 values varied between 3 ppm for *Allorchestes compressa* [29] and >175 ppm for *Boekosimus* sp. [30], but no data for *C. volutator* were reported. Mollusc (adults) LC50 values were in the range 33.8 ppm for the sand snail *Polinices conicus* [29] to 2,500 ppm for the scallop *Argopecten irradians* [31]; however, no data for *Mytilus edulis* were reported. The class Anthozoa (anemones and true corals) were not represented in the reviewed papers and the only seagrass was *Thalassia testudinum* with a 96-h LC50 of 200 ppm [32]. In general, the reviewed data show that Corexit 9527 is of low acute toxicity to most species, although embryo and larval stages are more sensitive. The results from this study are consistent with the literature data but suggest an order of sensitivity, based on NOEC and LOEC values, of: Anthozoa > macrophyte > crustacean > mollusc for both Corexit 9527 and SD-25, although the order of sensitivity partly is due to the differences in test methodology and endpoints.

Both dispersants caused a sharp increase in mortality over a narrow concentration range; this was most pronounced for Corexit 9527. Therefore, it is of more use to refer to NOEC or LOEC than LC50 values. The NOEC values for *A. viridis* were much lower than that observed for the other species tested (Table 1) and the sensitivity of the anemones was similar to that of the embryo or larval stages of molluscs and fish [33,34]. Of the anemones with extended tentacles that were unresponsive to stimuli following 48-h dispersant exposure of 20 ppm and, therefore, classed as moribund (Fig. 4a), all were able to recover when placed in clean seawater (Fig. 4b). All of the SD-25–exposed anemones also were able to recover from the 30-ppm exposure, but this was true of only 11% of Corexit-exposed anemones. The ability of the organisms to recover implies that the dispersants act reversibly on the neural receptors at low concentrations but cause irreversible membrane damage at higher concentrations, consistent with reported surfactant toxicity modes of action [27,28]. No effect on zooxanthallae density was found and the cells appeared undamaged even when their host tissue was damaged severely. It is likely that the host tissue protected the plant cells and that, during short-term exposure, the algae do not migrate from their hosts as happens with coral bleaching episodes. Anemones have been used as a surrogate organism for symbiotic coral assemblages [17,18], although it is not known if the skeletal cup of reef-building corals would afford the same protection for the zooxanthallae as the anemone.

The *M. edulis* mortality data were very variable, but Corexit 9527 clearly was more toxic at low concentrations (Fig. 2a). The total percentage of SD-25–exposed mussels that were closed at the end of the 48-h exposure period increased from 44 to 58%, but Corexit-exposed closed mussels decreased from 67 to 51% with a corresponding rise in the percentage dead (Table 2). A very pronounced effect was observed in the feeding rates of mussels exposed to 50 ppm of both dispersants, with Corexit-exposed organisms' filtration rates reduced so much that they were not significantly greater than system blanks (Fig. 3). Although the highly reduced filtration rates could be a behavioral response to the dispersants, the mussels' failure to open within the 72-h recovery period following the

48-h concentration-response test (Table 2) suggests that the mussels may have been suffering physiological damage as they would be expected to recover from nonspecific narcosis. Although there was no sublethal component to the *C. volutator* test, it was observed that many individuals failed to burrow quickly when exposed to ≥175 ppm dispersants. It was impossible to tell if it was the swimming amphipods that died later, although these likely had a greater exposure than those that burrowed rapidly into the sediment. Briggs et al. [8] used turbidity caused by stressed *Corophium* as a measure of toxicity and found a good correlation between turbidity during the first 24 h of exposure and mortality after 10 d. The failure to burrow and increased swimming activity observed at moderate contamination levels, are most likely an escape behavioral response rather than a physiological response because they occur with a range of toxicants [8,35]. The dispersant concentrations in this study that caused the greatest turbidity were within the confidence limits of the calculated LC50 values (Table 1). Under test conditions where escape was impossible, the behavioral response not to burrow probably proved to be detrimental, but in the wild it may enable the organisms to escape from localized areas of high concentration.

Both dispersants disrupted the Photosystem II (PSII) apparatus, known to be a primary casualty during stress conditions [36], within the leaves of *Z. marina* and this was found to be largely irreversible, especially for Corexit 9527 (Fig. 5). The PI is a standard composite of several JIP-test parameters and has been shown to be a highly sensitive measure that is correlated strongly with other measurements of plant health [24]. Indeed, at concentrations ≥200 ppm Corexit 9527, it was evident by visual inspection that the leaves were damaged severely and the outer leaves were becoming detached. Leaves of *Z. marina* possess a thin cuticle [37] that may afford a degree of protection from the dispersants, although it is clear that 24 h was sufficient for photosynthesis to be affected. Algae and spores with less protection likely are more sensitive, as found by Singer et al. [34], exposing zoospores of giant kelp, *Macrocystis pyrifera*, to Corexit 9527.

Extrapolating the results from this and other studies suggests that the most sensitive organisms to dispersants are those with the least protective tissue or shell. As well as the spore, embryo, and larval stages, it is likely that unprotected organisms such as nudibranchs and seaslugs would be vulnerable and, therefore, extra care may need to be taken where rare or endangered species are present. Under current dispersant-use guidelines that exist in the United Kingdom and elsewhere [1,2], dispersant concentrations are unlikely to be sufficiently high to cause harm to aquatic organisms within open waters. Measured concentrations at sea are rare but Bocard et al. [38] reported a maximum concentration of 13 ppm. However, if dispersants are used or spilled accidentally within estuaries, enclosed bays, or shallow coral reefs, it may be possible for concentrations to become elevated long enough to cause mortality of sensitive species and stress to more tolerant animals and plants. Any harm to aquatic species as a result of dispersant use must be measured against the potential harm that may arise from the toxic or physical effects of oil. The difficult decision of when, where, and under what conditions to use dispersants will remain as long as oil is transported via the sea. Spill response agencies are better able to make such decisions when provided with relevant ecotoxicological data. To our knowledge, these are the first reported results concerning the toxicity of Superdispersant-25; it has shown that this dispersant generally is of lower toxicity than Corexit 9527 to a range of coastal species, although equivalent in terms of NOEC and LOEC values; also, at maximum concentrations likely to be found at sea, any sublethal effects upon organisms are more likely to be reversible.

Acknowledgement—This study was financed by the Maritime and Coastguard Agency, the Department for Environment Food and Rural Affairs, the Department of Trade and Industry, and Minerals Management Service Grant RP 480. The Photosynthesis Membrane model was derived using Biolyzer® software provided by R. Maldonado-Rodriguez, Bioenergetics Laboratory, University of Geneva, Geneva, Switzerland.

REFERENCES

1. Maritime and Coastguard Agency. 2002. *Contingency Planning for Marine Pollution Preparedness and Response: Guidelines for Ports, 2002.* Southampton, UK.
2. Etkin DS. 1998. Factors in the dispersant use decision-making process: Historical overview and look to the future. *Proceedings of the Twenty-First Arctic and Marine Oil Spill Program Technical Seminar.* Environment Canada, Ottawa, ON, pp. 281–304.
3. George-Ares A, Clark JR. 2000. Acute toxicity of two Corexit dispersants. *Chemosphere* 40:897–906.
4. U.S. National Research Council. 1989. *Using Oil Dispersants on the Sea.* National Academy, Washington, DC.
5. Limia JM, Raffaelli D. 1997. The effects of burrowing by the amphipod *Corophium volutator* on the ecology of intertidal sediments. *J Mar Biol Assoc UK* 128:147–156.
6. Raffaelli D, Milne H. 1997. An experimental investigation of the effects of shorebird and flatfish predation on estuarine invertebrates. *Estuar Coast Shelf Sci* 24:1–13.
7. Bat L, Raffaelli D. 1998. Sediment toxicity testing: A bioassay approach using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. *J Exp Mar Biol Ecol* 226:217–239.
8. Briggs AD, Greenwood N, Grant A. 2002. Can turbidity caused by *Corophium volutator* (Pallas) activity be used to assess sediment toxicity rapidly? *Mar Environ Res* 55:181–192.
9. Roddie BD, Thain JE. 2001. Biological effects of contaminants: *Corophium* sp. sediment bioassay and toxicity test. Techniques in Marine Environmental Sciences 28. International Council for the Exploration of the Sea, Copenhagen, Denmark.
10. Center for Environment, Fisheries, and Aquaculture Science. 2001. Monitoring and surveillance of nonradioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1998. Aquatic Environment Monitoring Report 53. Center for Environment, Fisheries, and Aquaculture Science, Lowestoft, UK.
11. Conradi M, Depledge MH. 1998. Population responses of the marine amphipod *Corophium volutator* (Pallas, 1766) to copper. *Aquat Toxicol* 44:34–45.
12. Ciarelli S, Vonck WAPMA, van Stralen NM. 1997. Reproducibility of spiked-sediment bioassays using the marine benthic amphipod, *Corophium volutator*. *Mar Environ Res* 43:329–343.
13. Goldberg ED. 1975. The Mussel Watch—a first step in global marine monitoring. *Mar Pollut Bull* 6:111–113.
14. Galloway TS, Sanger RC, Smith KL, Fillmann G, Readman JW, Ford TE, Depledge MH. 2002. Rapid assessment of marine pollution using multiple biomarkers and chemical immunoassays. *Environ Sci Technol* 36:2219–2226.
15. Widdows J, Donkin P, Brinsley MD, Evans SV, Salkeld PN, Franklin A, Law RJ, Waldock MJ. 1995. Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. *Mar Ecol Prog Ser* 127:131–148.
16. Connor DW, Brazier DP, Hill TO, Northen KO. 1997. *Marine Nature Conservation Review: Marine Biotope Classification for Britain and Ireland,* Vol 1—Littoral Biotopes. Version 97.06, Report 229. Joint Nature Conservation Committee, Peterborough, UK.
17. Morrall CE, Galloway TS, Trapido-Rosenthal HG, Depledge MH. 2000. Characterization of nitric oxide synthase activity in the tropical sea anemone *Aiptasia pallida*. *Comp Biochem Physiol B* 125:483–491.
18. Whitehead LF, Douglas AE. 2003. Metabolite comparisons and

the identity of nutrients translocated from symbiotic algae to an animal host. *J Exp Biol* 206:3149–3157.
19. Davison DM, Hughes DJ. 1998. *An Overview of Dynamics and Sensitivity Characteristics for Conservation Management of Marine SACs,* Vol I—Zostera Biotopes. UK Marine SACs Project. Scottish Association for Marine Science, Argyll, UK.
20. Vergeer LHT, Aarts TL, deGroot JD. 1995. The wasting disease and the effect of abiotic factors (light-intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. *Aquat Bot* 52:35–44.
21. Scarlett A, Donkin P, Fileman TW, Evans SV, Donkin ME. 1999. Risk posed by the antifouling agent Irgarol 1051 to the seagrass *Zostera marina*. *Aquat Toxicol* 45:159–170.
22. Donkin P, Widdows J, Evans SV, Worrall CM, Carr M. 1989. Quantitative structure activity relationships for the effect of hydrophobic organic chemicals on rate of feeding by mussels (*Mytilus edulis*). *Aquat Toxicol* 14:277–294.
23. Strasser RJ, Srivastava A, Govindjee B. 1995. Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteri. *Photochem Photobiol* 61:32–34.
24. Hermans C, Smeyers M, Rodriguez RM, Eyletters M, Strasser RJ, Delhaye JP. 2003. Quality assessment of urban trees: A comparative study of physiological characterization, airborne imaging, and on-site fluorescence monitoring by the OJIP-test. *J Plant Physiol* 160:81–90.
25. Canevari GP. 1971. Oil slick dispersants and methods. Patent 3,793,218. U.S. Patent Office, Washington, DC. As cited in Scelfo GM, Tjeedema RS. 1991. A simple method for determination of Corexit 9527® in natural waters. *Mar Environ Res,* 31:69–78.
26. Ayles Fernie International. 2003. Superdispersant-25 Safety Data Sheet. Kent, UK.
27. Wells PG. 1984. The toxicity of oil spill dispersants to marine organisms: A current perspective. In Allen TE, ed, *Oil Spill Chemical Dispersants: Research, Experience, and Recommendations.* STP 840. American Society for Testing and Materials, Philadelphia, PA, pp 177–202.
28. Borseth JF, Aunaas T, Denstad JP, Nordtug T, Olsen AJ, Schmid R, Skjaervo G, Zachariassen KE. 1995. Transmembrane sodium energy gradient and calcium content in the adductor muscle of *Mytilus edulis* L. in relation to the toxicity of oil and organic chemicals. *Aquat Toxicol* 31:263–276.
29. Gulec I, Leonard B, Holdway DA. 1994. Oil and dispersed oil toxicity to amphipods and snails. *Spill Science & Technology Bulletin* 4:1–6.
30. Foy MG. 1982. Acute lethal toxicity of Prudhoe Bay crude oil and Corexit 9527 to Arctic marine fish and invertebrates. Technology Development Report EPS 4-EC-82-3. Environment Canada, Ottawa, ON.
31. Ordsie CJ, Garofalo GC. 1981. Lethal and sublethal effects of short-term acute doses of Kuwait crude oil and a dispersant Corexit 9527 on bay scallops, *Argopecten irradians*, and two predators at different temperatures. *Mar Environ Res* 5:195–210.
32. Baca BJ, Getter CD. 1984. The toxicity of oil and chemically dispersed oil to the seagrass *Thalassia testudinum*. In Allen TE, ed, *Oil Spill Chemical Dispersants: Research, Experience, and Recommendations.* STP 840. American Society for Testing and Materials, Philadelphia, PA, pp 314–323.
33. Singer MM, Smalheer DL, Tjeerdema RS, Martin M. 1990. Toxicity of an oil dispersant to the early life stages of four Californian marine species. *Environ Toxicol Chem* 9:1389–1397.
34. Singer MM, Smalheer DL, Tjeerdema RS, Martin M. 1991. Effects of spiked exposure to an oil dispersant on the early life stages of four marine species. *Environ Toxicol Chem* 10:1367–1374.
35. Kravitz MJ, Lamberson JO, Ferraro SP, Swartz RC, Boese BL, Specht DT. 1999. Avoidance response of the estuarine amphipod *Eohaustorius estuarius* to polycyclic aromatic hydrocarbon–contaminated, field-collected sediments. *Environ Toxicol Chem* 18:1232–1235.
36. Morales F, Belkhodja R, Abadia A, Abadia J. 2000. Photosystem II efficiency and mechanisms of energy dissipation in iron-deficient, field-grown pear trees (*Pyrus communis* L.). *Photosynth Res* 63:9–21.
37. Larkum AWD, Roberts G, Kuo J, Strother S. 1989. Gaseous movement in seagrasses. In Larkum AWD, McComb AJ, Shepherd SA, eds, *The Biology of Seagrasses with Special References to the Australian Region.* Elsevier, Amsterdam, The Netherlands, pp 686–722.
38. Bocard C, Castaing G, Gatellier C. 1984. Chemical oil dispersion in trials at sea and in laboratory tests: The key role of dilution processes. In Allen TE, ed, *Oil Spill Chemical Dispersants: Research, Experience, and Recommendations.* STP 840. Philadelphia, PA, pp 125–142.