The effects of oil and oil dispersants on the amphipod *Gammarus oceanicus* from Arctic waters

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Amphipods of the species *Gammarus oceanicus* were exposed to water soluble fractions and water emulsions of Statfjord A+B crude oil, the dispersants Finasol OSR-5 and Finasol OSR-12, and combinations of the oil and dispersants. Adding Finasol OSR-12 to the crude oil caused a reduction in the mortality of the amphipods compared with amphipods exposed to the water soluble fraction and the water emulsion of crude oil alone, probably due to a reduced mole fraction of toxic oil components in the mixture of oil and dispersant. Exposure to sublethal concentrations of water soluble fractions increased the respiratory rates of the amphipods in the majority of the exposed groups. The water soluble fractions slightly increased the concentrations of sodium in the haemolymph and in the whole organism. Some exposures gave a significant increase in the relative water content of the amphipods. The water soluble fractions probably increase the membrane permeability to water and ions, leading to an increased influx of water and sodium from the medium. The increased respiratory rates are likely to be due to a compensatory extrusion of sodium. Exposure to sublethal concentrations of water emulsions reduced the respiratory rates of the amphipods, probably due to oil droplets adhering to the gill membranes and thus causing a reduced rate of oxygen diffusion into the organisms. The majority of the exposures to water emulsions increased concentrations of sodium in the haemolymph as well as in whole organisms. Thus, sodium probably accumulates in the intracellular compartments because the sodium pumps are restricted by the reduced energy available. This is likely to lead to an osmotic swelling of cells. Reduced total free amino acid concentrations in these amphipods is ascribed to volume regulation of the swollen cells, and a reduced co-transport of sodium and free amino acids from the haemolymph to the intracellular compartments.

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Introduction

Since some processes in the Arctic occur slowly, the ecosystems are more vulnerable to environmental impacts than in temperate areas. Investigations of the chemical behaviour of oil in the Arctic have revealed that oil in the water and oil embedded in the sediments degrade slowly and can remain close to their original state for long periods in the ecosystem (Atlas 1977; Atlas & Busdosh 1976; Atlas et al. 1978). Marine organisms in tidal zones subject to oil spills can thus be chronically exposed to oil components over a long period of time.

Oil drilling at sea and transportation of oil by tankers will always involve the possibility of an oil spill. An oil spill may affect the marine environment in several ways. When the negative effects of the oil spill are so comprehensive that chemical treatment is desirable to limit the distribution of the slick and to enhance the degradation of the oil components, the addition of more chemicals to the environment may cause environmental impacts beyond those caused by the oil spill alone. Hence, the decision as to how to deal with an oil spill must be based on evaluation of the effects of the oil spill alone in relation to the effects of treating it with chemicals.

Oils are mixtures of a variety of complex polar and nonpolar chemical compounds with differing degrees of toxicity. Østgaard & Jensen (1983) pointed out that, depending on the energy input in the system, oil exists in two different forms in seawater: 1) as fractions which are physically dissolved in the water (water soluble fractions) or 2) as an emulsion with droplets dispersed in the water (water emulsion). An untreated oil slick undergoes qualitative and quantitative changes that are likely to alter its toxicity; drift and spreading of the slick may subject various categories of marine organisms to exposure to the toxic

components. Chemical treatment of an oil slick influences the qualitative and quantitative changes of the oil as well as drift and spreading. In addition comes the possible toxicity of the chemicals used.

The most feasible approach in predicting the effects of pollutants is to focus on the causal or mechanistic relationship between the presence of a pollutant and its biological consequences. The effects of a pollutant on population and ecosystem levels are always secondary to the effects of the pollutant on biological processes in individual organisms. Lethal or sublethal effects of pollutants are always caused by a displacement of one or more regulated physiological parameters beyond the tolerated limits. Thus, studies of the causal relationships between a pollutant and its environmental effects have to deal with effects on physiological parameters. The principal value of physiological parameters is their capacity to describe the condition of the organism. In addition they may provide information about the mechanisms of action of the toxicants.

A number of investigations have shown that oil may be toxic to marine organisms (Malins 1977; Engelhardt 1985). The influence of oils and chemicals on biological membranes is well established, but their mechanistic action has not been studied in great detail. Thus, very little is known about the effects of such compounds on the physiological mechanisms involved in the cell functions, and little effort has been made to distinguish between the effect of the fractions of the oil which are dissolved in the water (water soluble fraction) and the effects of the oil droplets in the water (water emulsion).

Respiratory rates are variable and may be affected by many factors, including the presence of oils and chemicals (Malins 1977). Percy (1977) demonstrated that the respiratory rates of the amphipod *Onisimus (Boeckosimus) affinis*, from the Beaufort Sea, were reduced by low concentrations of crude oil in the seawater, but increased by high concentrations. In general, petroleum concentrations of at least 1 mg l^{-1} are required for effects to be visible in juvenile and adult crustaceans and molluscs (Malins 1977).

Gammarus oceanicus and sympagic amphipods in the Arctic are known to be effective osmoregulators (Aarset & Aunaas 1987, 1990; Aunaas et al. 1989). Exposed to high osmolalities (>800 mOsm) such organisms are slightly hyperosmotic to the medium. However, the regulation of transmembrane concentration differences of solutes is important in order to secure optimal cellular functions even at isosmotic conditions. Sodium and free amino acids (FAAs) are of particular importance among the regulated solutes.

Sodium is extruded from animal cells by an active transport mechanism (Na-K-dependent ATPase). This active extrusion of sodium gives rise to a high extracellular and low intracellular concentration of sodium in all animals. Contrary to what should be expected from pure thermodynamic estimates, substantial experimental evidence indicates that the active extrusion of sodium from resting frog muscle cells requires a great fraction (up to 75%) of the total cellular energy turnover (Florey 1966). Active insects, crustaceans, and vertebrates expend one-third of their metabolic energy for osmotic and ionic regulation at cell membranes while the energy used at interfaces with the environment is less (Prosser 1986). Evidence from studies on effects of low pH and aluminium ions on fish indicates that the sodium pumping in the gill epithelium is strongly affected by chemical pollution, and that this and related effects are responsible for the harmful effects of chemical pollution in fish (Leivestad 1982; Reite & Staurnes 1987).

Aarset & Zachariassen (1983) found that the electrochemical potential difference of sodium as well as the intracellular concentrations of free amino acids in blue mussels (*Mytilus edulis*) are affected by oil and oil treating chemicals. Free amino acids (FAAs) are transported into the cells by a co-transport with sodium. Sodium moves energetically downhill into the cells together with an uphill movement of the FAAs. The majority of the more than 20 different FAAs and amino acid-like substances that occur in animals are accumulated intracellularly by sodium-dependent mechanisms. Consequently, FAAs occur in considerably higher concentrations in intracellular than in extracellular compartments.

The gill membranes of marine amphipods are in intimate contact with the medium. Thus, physical and physiological processes located to the gill membranes are likely to be affected by oils and chemicals in the water. The purpose of this study has been to investigate the effects of water soluble fractions and water emulsions of crude oil and dispersants on mortality and mechanisms involved in the distribution of inorganic ions and free amino acids in the amphipod *Gammarus oceanicus* from the tidal zone in Arctic waters.

Materials and methods

Gammarus oceanicus were collected from the tidal zone in Kongsfjorden, Svalbard, during the period 1985–1987. Experiments were performed on animals in laboratories at Ny Ålesund, Svalbard, and on animals transported to the laboratories in Trondheim. The transportation of amphipods from Svalbard to Trondheim took place in aerated thermo containers. The animals were stored in running seawater at 5°C in the laboratory for 24–48 hours prior to the experiments. Only intermolt animals (Charniaux-Legrand 1952) ranging from 18–44 mm in size, measured from rostrum to the base of the telson, were used in the experiments.

The amphipods were exposed for 96 hours at 5°C in glass aquaria to sublethal concentrations of the water soluble fractions and water emulsions. In the experiments with water soluble fractions exposure solutions were renewed every 24 hours during the exposure period. Amphipods exposed to pure filtered seawater at 875 or 1010 mOsm and 1010 mOsm served as controls in the experiments with water soluble fractions and water emulsions, respectively. No stirring, apart from the activity created by the amphipods themselves, was applied in the experiments with water soluble fractions.

Stock solutions of water soluble fractions of Statfjord A+B crude oil, the dispersants Finasol OSR-5 and OSR-12, and combinations of oil and chemicals were prepared according to a method described by Østgaard & Jensen (1983). The oil and chemicals were carefully applied on the surface of 8-l filtered (pore size 0.45 µm), 34.5‰ seawater in glass flasks. The flasks were then sealed. A magnetic stirrer at the bottom of each flask was rotating at low speed (appr. 30 rpm) for six days. The stirring did not affect the interface between oil and water. The preparations took place in darkness at 5°C. After six days, the stirring was terminated, and the oil-water system allowed to settle for 24 hours. The water phase (stock solution) was then transferred to 11 dark bottles, wrapped in aluminium foil, and stored at 5°C.

In the preparation of stock solutions 400 ml of the crude oil, 400 ml of Finasol OSR-12 and 40 ml of Finasol OSR-5 were added to 8 l seawater. The combinations of oil and chemicals were premixed for 1 min by the use of a stirrer (1100 rpm) in 2 l beakers. The amounts of crude oil and chemicals used were 400 ml oil + 400 ml Finasol OSR-12 and 400 ml oil + 40 ml Finasol OSR-5 according to the ratios of oil/chemical recommended by the producer of the chemicals. The stock solutions and samples from an experimental set up simulating an exposure to diluted stock solutions were analysed chemically for volatile hydrocarbons (C_2-C_{10}). The stock solutions were also analysed for the neutral and acidic fractions of the "Total Extractable Organic Matter" (TEOM, C_{10}–C_{36}) which were determined by gas chromatographic methods (Leistad et al. 1989). The quantitative results from the different chromatographic analyses applied to the stock solutions are summarised in Table 1.

Water emulsions were made by a modified version of a method described by Blackman et al. (1977). This method, which is described by Olsen et al. (1989), involves exposure of the amphipods to oil and dispersants continuously stirred into the seawater at 1400 rpm. The arrangement prevents the animals from entering the moving parts of the equipment and from sticking to the surface of the seawater in the exposure aquarium.

Prior to the sublethal experiments, the amphipods were exposed to different dilutions of the stock solutions of the water soluble fractions and

Table 1. Quantitative results from different chromatographic analyses applied to the stock solutions used in the experiments with water soluble fractions. Concentrations are given in mg l^{-1}. (From Leistad et al. 1989).

Sample	Average C_{10}–C_{36} TEOM	Sum (gas equil.) C2–C8 hydrocarbons	Sum (DCM extract) C_6–C_3- Benzenes	Non-identified compounds
Statfjord A+B crude oil	1.5	22.6	16.4	0
Finasol OSR-5	200.0	0.005	0	30.0
Finasol OSR-12	180.0	0.1	0	45.0
Crude oil + OSR-5	80.0	26.0	12.0	46.0
Crude oil + OSR-12	100.0	15.0	1.9	42.0

to different concentrations of water emulsions to establish lethality curves. The sublethal concentrations, chosen on basis of these curves, were in the concentration range giving 0–10% lethality in the exposed groups. The percentages of stock solutions used in the sublethal exposure experiments with water soluble fractions were 20% for crude oil, 10% for OSR-5 and the crude oil + OSR-5, and 100% for OSR-12 and crude oil + OSR-12. The concentrations used in the sublethal exposure experiments to water emulsions were 30 mg crude oil l^{-1} seawater, 20 mg Finasol OSR-5 l^{-1} seawater, 300 mg Finasol OSR-12 l^{-1} seawater, 200 mg crude oil + 20 mg OSR-5 l^{-1} seawater and 100 mg crude oil + 100 mg OSR-12 l^{-1} seawater.

The respiratory rates of *G. oceanicus* were measured by using a modified version of a constant pressure respirometric method (Engelmann 1963). Each respirometer contained one individual immersed in 5 ml filtered (0.2 μm) seawater with the same osmolality as in the exposure solution. In order to keep a constant temperature (5 ± 0.05°C), the respirometers were immersed in fresh water in a cooling incubator. Respirometers with 5 ml filtered seawater were used as blanks. The incubation period in the respirometer was 30 min, after which 6 to 8 measurements (accuracy within ± 1%) were made at 10–15 min intervals. The standard respiratory rates were then estimated. The specific rates of oxygen consumption were calculated in relation to the dry body mass of the animals and at standard temperature, pressure, and dry conditions (STPD). The dry mass of the amphipods was obtained after drying for 24 hours at 105°C.

The wet mass of individual amphipods from the exposed and control groups was determined, and the animals were transferred to test tubes with 4 ml 0.2N HNO_3 in order to determine the total concentration of sodium in the animals (Lutz 1972). The relative water content was determined by gravimetry on a parallel group of animals after drying the specimens for 24 hours at 105°C.

Samples of haemolymph were obtained by inserting a pointed glass capillary, containing a small quantity of paraffin-oil, dorsally through the intersegmental membrane. After a sample (10 to 40 μl) was taken, the capillary was sealed at one end by melting in a flame and centrifuged on a Compur 1100 micro-centrifuge, leaving the haemolymph sample isolated under a layer of paraffin-oil (Zachariassen et al. 1982). The capillaries were stored frozen at −27°C for up to 3 weeks prior to the analyses. The concentrations of sodium in the haemolymph and in the acid extracts were determined on a Perkin Elmer AAS 300 atomic absorption spectrophotometer.

For analyses of FAAs, whole specimens (wet weight 500–800 mg) were homogenised in 4 ml 5% sulfosalicylic acid to precipitate proteins. The supernatant was analysed for individual FAAs on a Varian Vista 5500 high performance liquid chromatograph (HPLC) equipped with a Rheodyne Model 7126 automatic sample injector, a Varian Fluorichrom™ fluorescence detector, and a Varian 4270 integrator. A Supelco Supelcosil LC 18, 3 μm reversed phase (5-8278) column was selected and the samples were injected manually. The procedure described by Lindroth & Mopper (1979) involved *o*-phtaldialdehyde as a derivating agent and a gradient eluent system with methanol and an acetate buffer (pH = 5.9).

Due to rather small sample sizes in the experimental groups, the underlying population was assumed to be normally distributed and the Student's t-test was used to test the statistical significance of the results.

Results

Fig. 1 shows the time dependence of the total C_2–C_8 hydrocarbon concentrations for crude oil and the combination of crude oil and the dispersants Finasol OSR-5 and OSR-12 in test aquaria with stock solutions prepared as water soluble fractions and diluted by 7 parts seawater. The results indicate that evaporation of volatile components during the experiment caused the concentrations to drop by about 30% from the initial concentration during the first 24 hours.

The mortality of groups of *G. oceanicus* exposed to dilutions of the water soluble fractions of oil and dispersants is shown in Fig. 2. The results show that the water soluble fraction of Finasol OSR-5 induced the highest mortality, followed by the crude oil combined with this dispersant. The water soluble fraction of the crude oil alone was less toxic, but high mortality was registered in groups exposed to high concentrations of the stock solution. Water soluble fractions of crude oil in combination with Finasol OSR-12 and of Finasol OSR-12 alone produced moderate mortality even in groups exposed to 100% of the stock solutions.

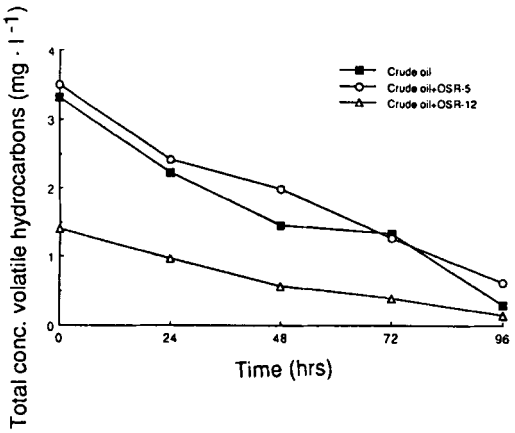

Fig. 1. Time course in the loss of total C2–C8 hydrocarbons (mg l^{-1}) in diluted (1:7) stock solutions of water soluble fractions of Statfjord A+B crude oil and of Statfjord A+B crude oil in combination with the dispersants Finasol OSR-5 and OSR-12 from exposure aquaria with no amphipods present. The analyses were performed by gas phase equilibration/gas chromatography. (From Leistad et al. 1989).

The mortality of groups of amphipods exposed to the water emulsions of the crude oil, the dispersants, and the combinations of oil and dispersants is shown in Fig. 3. A mortality of about 50% was registered in the group exposed to 100 mg l^{-1} of the crude oil with increasing mortality at higher concentrations. The effects of concentrations from 0 to 100 mg l^{-1} were not tested, but the slope of the mortality curve indicates that there may be significant mortality even at low concentrations. For higher concentrations the mortality of the groups exposed to the combination of the crude oil and Finasol OSR-5 is close to that of the oil alone. No mortality was registered in the groups exposed to 0 to 30 mg l^{-1} of Finasol OSR-5, but the mortality increased markedly in the groups of amphipods which were exposed to concentrations of 40 mg l^{-1} and higher. There was low mortality in groups of amphipods exposed to Finasol OSR-12, and 30% mortality was registered following exposure to

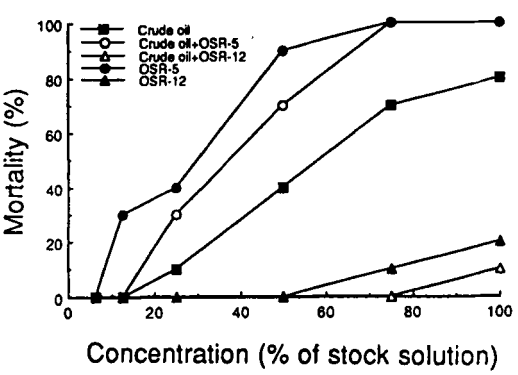

Fig. 2. Mortality (%) of groups of 20 individuals of *Gammarus oceanicus* exposed for 96 hours at 5°C to water soluble fractions of Statfjord A+B crude oil, the dispersants Finasol OSR-5 and OSR-12 and the combination of oil and dispersants. The exposure concentrations are given in % of the original stock solutions listed in Table 1.

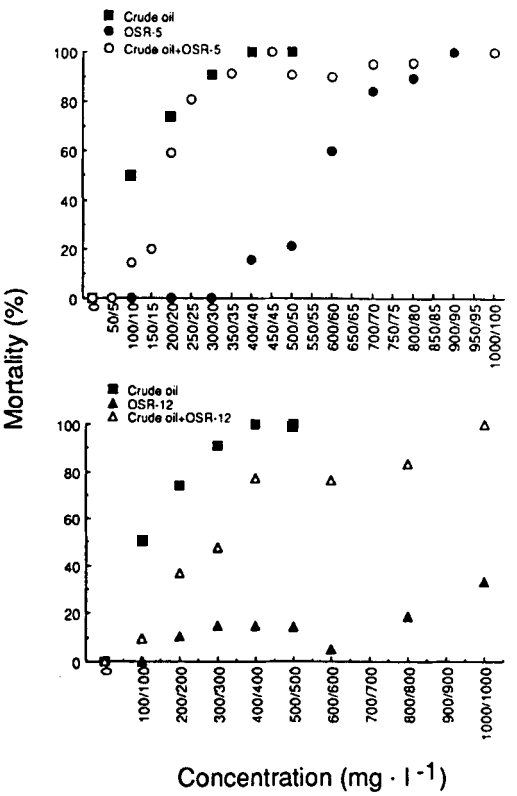

Fig. 3. Mortality (%) of groups of 20 individuals of *Gammarus oceanicus* exposed for 96 hours at 5°C to (upper graph) water emulsions of Statfjord A+B crude oil, and dispersant Finasol OSR-5, and the combination of oil and dispersant, and (lower graph) to water emulsions of Statfjord A+B crude oil, the dispersant Finasol OSR-12, and the combination of the oil and the dispersant. The exposure concentrations indicated on the abcissa represent the initial amounts (mg l^{-1}) of components added to the seawater. The lower figures on the abscissa represent the initial amounts (mg l^{-1}) of oil added to the seawater, alone or in the combination with dispersants. The upper figures on the abscissa represent the amounts (mg l^{-1}) of dispersants added to the seawater, alone or in the combination with crude oil.

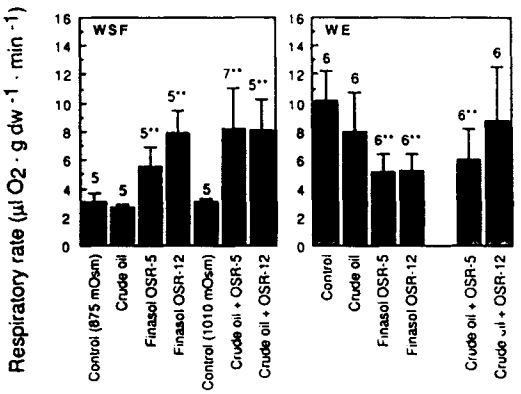

Fig. 4. Respiratory rates of *Gammarus oceanicus* (mean ± SD) at 5°C exposed to water soluble fractions (WSF) and water emulsions (WE) of Statfjord A+B crude oil, the dispersants Finasol OSR-5 and OSR-12, and the combinations of oil and dispersants. The exposures to water soluble fractions involve two groups of experiments at different osmolalities, 875 and 1010 mOsm, as indicated in the control legends. The values for exposed groups are given on the right hand side of the corresponding control value. The number of individuals in each group is indicated above the bars. The stars indicate the significance level (* $p \leq 0.05$ and ** $p \leq 0.01$).

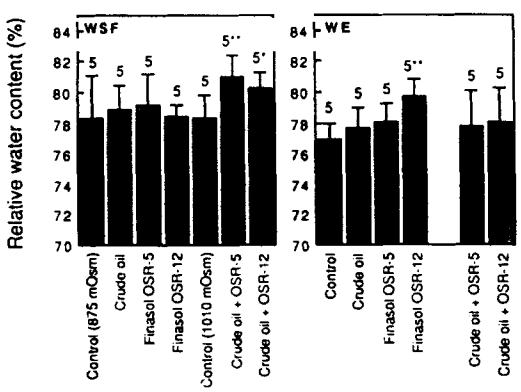

Fig. 5. Relative water content in groups of *Gammarus oceanicus* (mean ± SD) at 5°C exposed to water soluble fractions (WSF) and water emulsions (WE) of Statfjord A+B crude oil, the dispersants Finasol OSR-5 and OSR-12, and the combinations of oil and dispersants. The exposures to water soluble fractions involve two groups of experiments at different osmolalities, 875 and 1010 mOsm, as indicated in the control legends. The values for exposed groups are given on the right hand side of the corresponding control value. The number of individuals in each group is indicated above the bars. The stars indicate the significance level (* $p \leq 0.05$ and ** $p \leq 0.01$).

1000 mg l^{-1} of this dispersant. The results show that mortality in the groups exposed to the combination of crude oil and Finasol OSR-12 were higher than in groups exposed to the dispersant alone, and lower than in the groups exposed to the oil alone at corresponding concentrations.

Fig. 4 shows the respiratory rates of *G. oceanicus* exposed to sublethal concentrations of water soluble fractions and water emulsions of the oil,

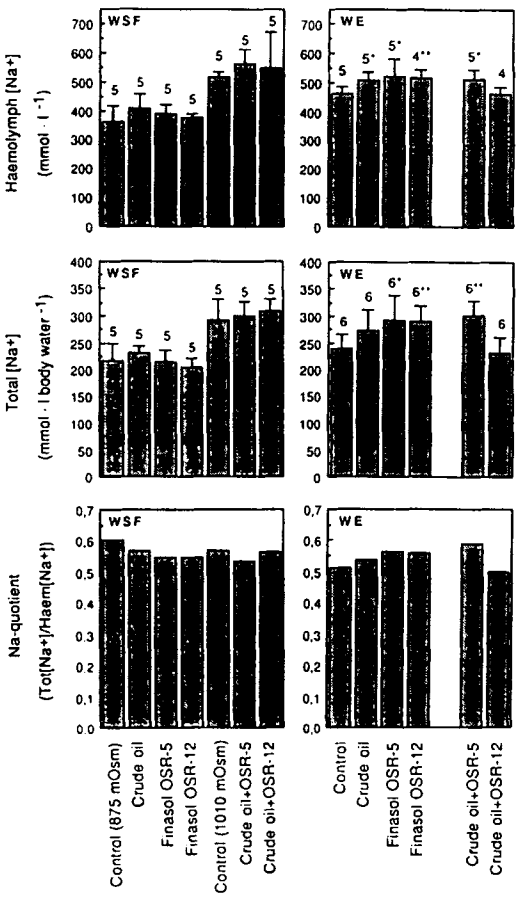

Fig. 6. Haemolymph (upper) and total (middle) concentrations of sodium and the quotient between total and haemolymph concentrations of sodium (lower) in *Gammarus oceanicus* (mean ± SD) at 5°C exposed to water soluble fractions (WSF) and water emulsions (WE) of Statfjord A+B crude oil, the dispersants Finasol OSR-5 and OSR-12, and the combinations of oil and dispersants. The exposures to water soluble fractions involve two groups of experiments at different osmolalities, 875 and 1010 mOsm, as indicated in the control legends. The values for exposed groups are given on the right hand side of the corresponding control value. The number of individuals in each group is indicated above the bars. The stars indicate the significance level (* $p \leq 0.05$ and ** $p \leq 0.01$).

dispersants and combinations of oil and dispersants. The results show a significant increase in the respiratory rates of amphipods which were exposed to water soluble fractions of Statfjord A+B crude oil in combination with the dispersants and in amphipods exposed to the water soluble fractions of the dispersants alone. Exposure to water soluble fractions of crude oil had no effect on oxygen consumption. The results indicate that the respiratory rates were reduced in amphipods exposed to water emulsions of crude oil, dispersants and crude oil in combination with Finasol OSR-5.

The relative water contents of *G. oceanicus* exposed to the water soluble fractions and water emulsions are presented in Fig. 5. A significant increase in the relative water content was registered in amphipods exposed to water soluble fractions of both combinations of oil and dispersants and in amphipods which were exposed to the water emulsion of Finasol OSR-12. In groups of amphipods exposed to the other water soluble fractions and water emulsions there were small, but not significant increases in the relative water content when compared to the controls.

Fig. 6 shows the total concentrations and con-

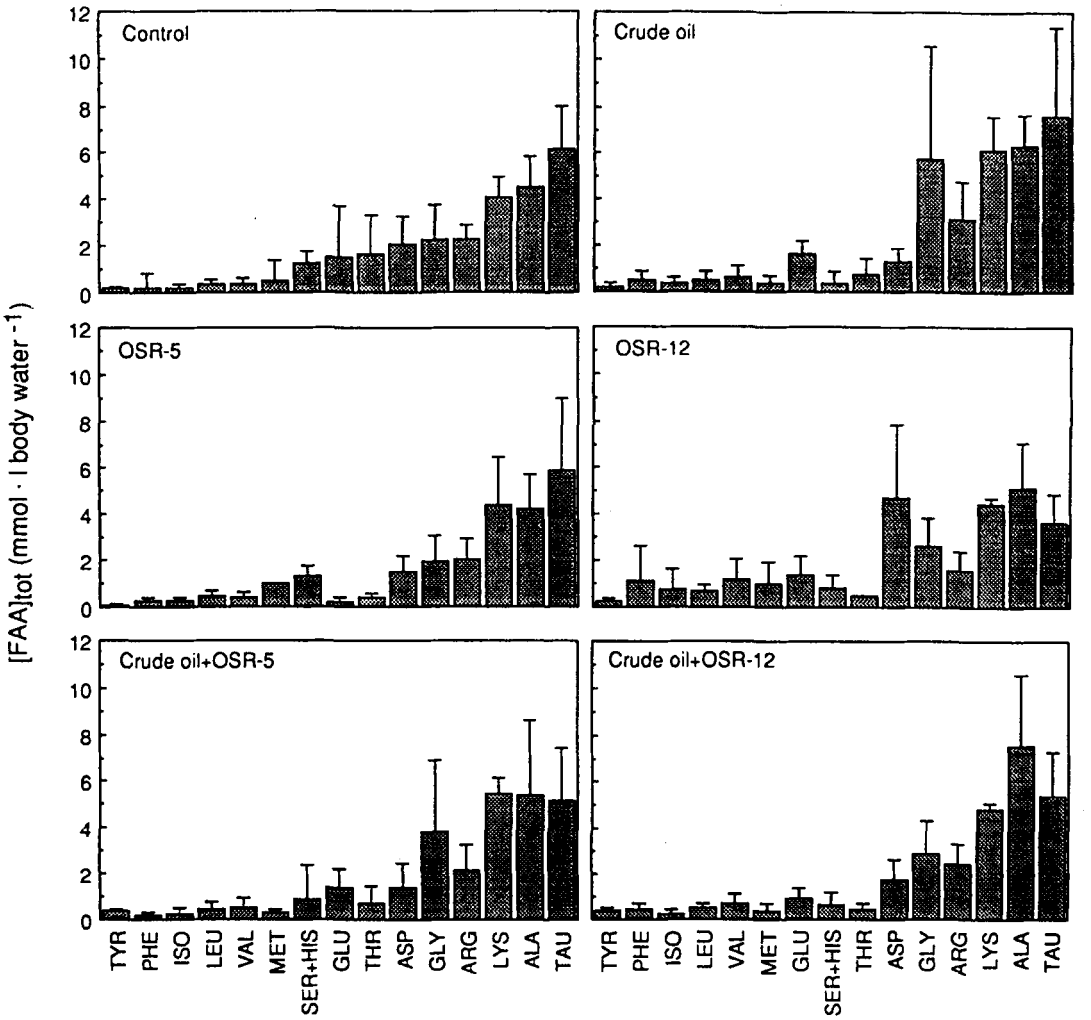

Fig. 7. Total concentrations of individual free amino acids (FAAs) in *Gammarus oceanicus* (mean ± SD) exposed to water soluble fractions of Statfjord A+B crude oil, the dipersants Finasol OSR-5 and OSR-12, and the combinations of oil and dispersants at 5°C in 1010 mOsm seawater. The number of individuals in each group is 5. Significance levels between exposed groups and the control are shown in Fig. 9.

centrations of sodium in the haemolymph of *G. oceanicus* exposed to water soluble fractions and water emulsions of oil and chemicals and the quotient between the total and the haemolymph concentration of sodium in the organisms. The results indicate that there was a slight increase in both the total sodium concentrations and the haemolymph concentrations in the amphipods exposed to the water soluble fractions.

Increased total and haemolymph concentrations of sodium were registered in all groups of animals in experiments with water emulsions, except for in the group exposed to the water emulsion of crude oil in combination with Finasol OSR-12. The increase in total sodium concentration was significant in amphipods exposed to water emulsions of Finasol OSR-5, Finasol OSR-12, and in the group exposed to the crude oil in combination with Finasol OSR-5 ($p < 0.05$, $p < 0.01$ and $p < 0.01$, respectively). The increase in the haemolymph concentration was significant in amphipods exposed to water emulsions of Statfjord A+B crude oil, Finasol OSR-5, Finasol OSR-12, and in the group exposed to the crude

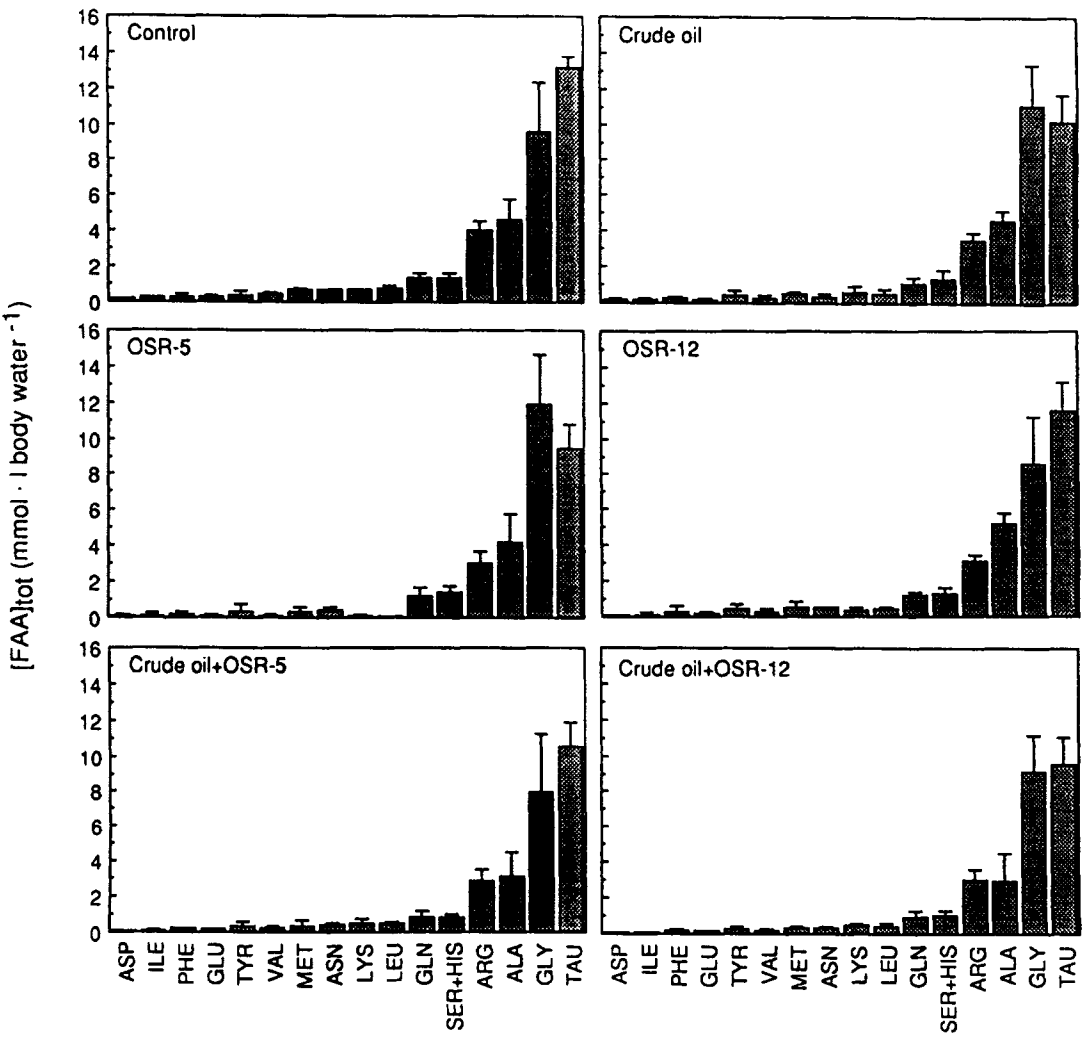

Fig. 8. Total concentrations of individual free amino acids (FAAs) in *Gammarus oceanicus* (mean ± SD) exposed to water emulsions of Statfjord A+B crude oil, the dipersants Finasol OSR-5 and OSR-12, and the combinations of oil and dispersants at 5°C in 1010 mOsm seawater. The number of individuals in each group is 5. Significance levels between exposed groups and the control are shown in Fig. 9.

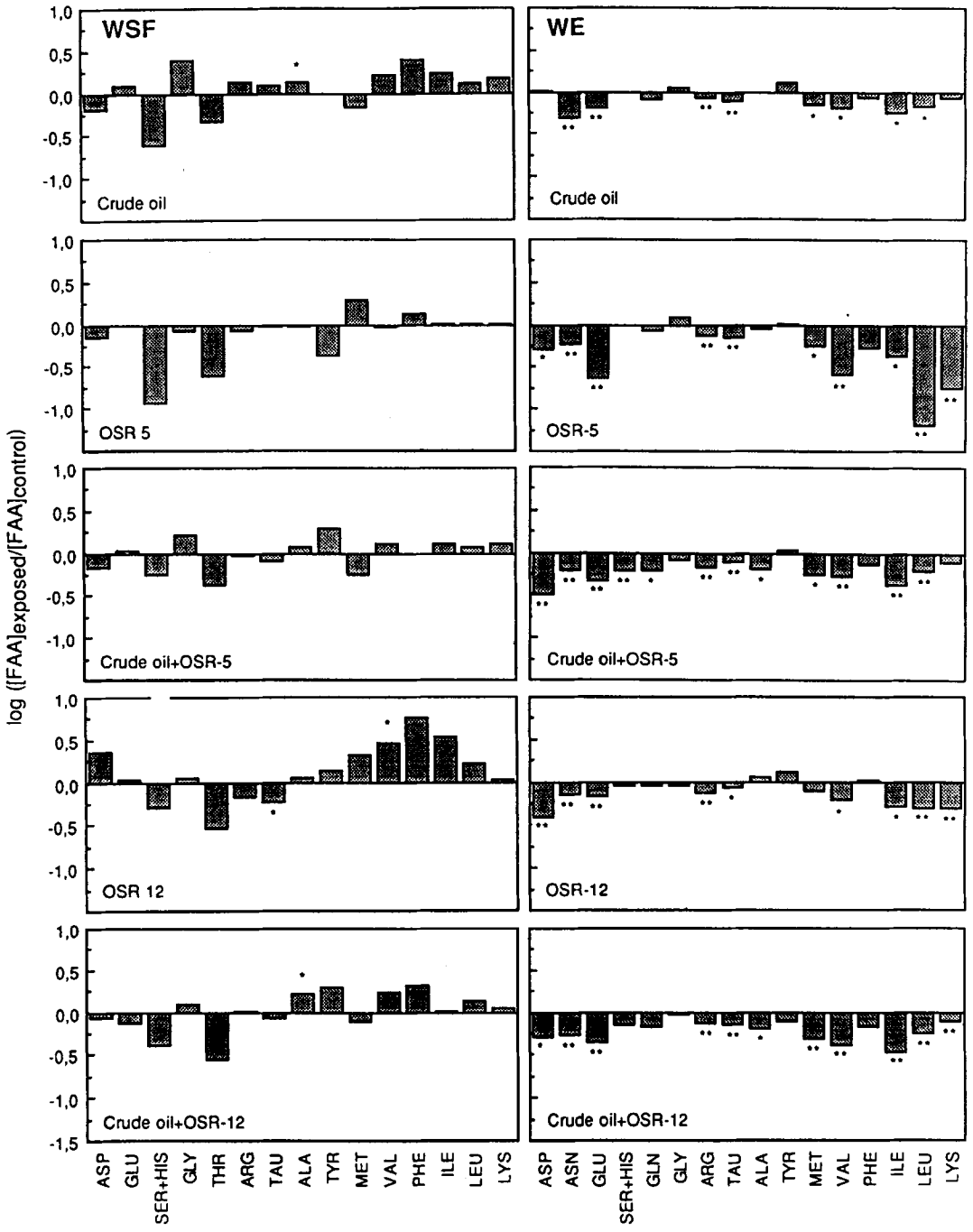

Fig. 9. Effects of water soluble fractions (WSF) and water emulsions (WE) of Statfjord A+B crude oil, the dispersants Finasol OSR-5 and OSR-12, and the combinations of oil and dipersants on the total, individual concentrations of free amino acids (FAAs) in *Gammarus oceanicus* at 5°C in 1010 mOsm seawater. The values are calculated from values in Figs. 4 and 5 and presented as $\log([FAA]_{exposed}/[FAA]_{control})$. The stars indicate the significance level (* $p \leq 0.05$ and ** $p \leq 0.01$).

oil in combination with Finasol OSR-5 ($p < 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively).

The quotient between total and haemolymph concentrations of sodium seems to be reduced in the groups exposed to water soluble fractions and increased in the groups exposed to water emulsions of crude oil, dispersants and the combination of oil and Finasol OSR-5. The combination of crude oil and Finasol OSR-12 does not seem to affect the sodium quotient neither as a water soluble fraction nor as a water emulsion.

The total concentrations of individual FAAs in amphipods exposed to water soluble fractions of oil and chemicals are presented in Fig. 7. No significant changes in the sum of the individual FAAs were observed in any of the exposed groups. However, as shown in Fig. 9, there is a significantly ($p < 0.05$) reduced concentration of taurine (TAU) in the group exposed to Finasol OSR-12. A significant ($p < 0.05$) increased concentration of valine (VAL) was observed in the same group. In the amphipods exposed to the water soluble fraction of crude oil there was a significant ($p < 0.05$) increase in alanine (ALA). A similar significant increase in alanine was observed in the amphipods exposed to the water soluble fraction of crude oil in combination with Finasol OSR-12. No significant changes in the concentrations of individual FAAs were observed in any of the two other exposed groups.

Fig. 8 shows the total concentrations of individual FAAs in amphipods exposed to water emulsions of oil and chemicals. A reduction in the sum of FAAs was observed in all exposed groups. The reduction was significant in the groups exposed to Finasol OSR-12 and crude oil in combination with Finasol OSR-5 and Finasol OSR-12 ($p < 0.05$, $p < 0.01$ and $p < 0.01$ respectively). As shown in Fig. 9, there are significant reductions in the majority of the individual FAAs in all groups of amphipods exposed to the different water emulsions.

Discussion

The oil dispersant Finasol OSR-5 is water soluble and the chemical analyses reveal that the water soluble fraction of this component contains high concentrations of organic compounds (Table 1). These compounds are likely to involve surface active agents. The high mortality observed in the group exposed to Finasol OSR-5 may therefore be an effect of relatively high concentrations of such components in the seawater. The water soluble fraction of crude oil mainly contains high concentrations of light aromatic and other light components of the oil (Table 1). Such components are supposed to be the most toxic constituents of the oil (Lee et al. 1972), and the toxicity is reflected in the high mortality in groups of amphipods exposed to even the highest dilutions of the stock solution. The dispersant Finasol OSR-12 is paraffin based and the majority of components dissolved in the water soluble fraction are long-chained (C_{10}–C_{36}) hydrocarbons which are supposed to be less toxic to organisms in the marine environment. This is reflected in the low mortality in amphipods exposed to even 100% of the stock solution of Finasol OSR-12.

The water soluble fraction of crude oil + Finasol OSR-12 caused a marked reduction in the mortality of the amphipods compared to those exposed to the water soluble fraction of crude oil alone. This may be due to a reduced mole fraction of the components from the oil in the oil-dispersant mixture used to prepare the stock solution. Exposure to the water emulsion involving a dispersion of the oil with Finasol OSR-12 also caused a marked reduction in the mortality of the amphipods compared to the exposure to the water emulsion to crude oil alone. This may be due to a reduced tendency of the dispersed oil to adhere to the surface of the gills. A reduced mole fraction of the oil components in the droplets containing high amounts of the paraffin based Finasol OSR-12 will also serve to reduce the tendency of oil components to enter the animals from the droplets. In contrast to Finasol OSR-12, Finasol OSR-5 does not influence the mole fraction of the oil components neither in the premixture prepared for the stock solutions in the experiments with water soluble fractions nor in the oil droplets formed in the dispersion in the experiments with water emulsions. Water soluble fractions involving a dispersion of the oil with Finasol OSR-5 increased the mortality compared to water soluble fractions of crude oil alone, probably due to toxic surfactants in the dispersant which were then added to the solution.

When exposed to high osmolality seawater (>800 mOsm), amphipods are normally slightly hyperosmotic to the environment (Aunaas et al. 1989). Thus, there will be an osmotic influx of water into the animals. Compared to seawater, haemolymph contains a large fraction of nega-

tively charged solutes such as FAAs and proteins, causing the organism to be negatively charged in relation to the seawater. The negatively charged inside of the amphipod will probably cause positively charged solutes to enter the organism from the seawater due to a Donnan-effect. Thus, despite the fact that the concentration of sodium in the body fluid is higher than in the medium, there is probably a passive net diffusion of sodium across gill membranes into the body fluid due to the electrical potential between the haemolymph and the environment.

In amphipods exposed to oil in combination with the chemicals and to the pure chemicals, an increased relative water content is registered (Fig. 2). The results indicate that the total and haemolymph sodium concentrations are slightly increased in the exposed groups (Fig. 3). This indicates that there has been a passive influx of sodium into the organisms, probably due to an increased permeability to sodium or a reduced active extrusion of sodium. An increased influx of sodium and the subsequent increased solute concentration in the haemolymph may cause an increased osmotic influx of water. This may explain the increased relative water content observed in the exposed amphipods.

An increased concentration of sodium in the haemolymph probably leads to an increased diffusion of sodium from the extracellular to the intracellular compartments. The cells are likely to respond to an increased intracellular concentration of sodium by a regulatory increase in cellular sodium extrusion. This may be at least part of the basis of the increased rates of oxygen consumption in the amphipods exposed to the water soluble fractions.

The oil droplets formed in the water emulsions probably adhere to the gill membranes and form a barrier to oxygen diffusion through the membranes. Reduced respiratory rates were registered in all groups exposed to water emulsions, except in the group exposed to crude oil in combination with OSR-12.

The majority of the exposures involving water emulsions were associated with increased concentrations of sodium in the haemolymph as well as in the whole organisms. The results also revealed that the quotients between total concentrations and haemolymph concentrations of sodium in amphipods exposed to the water emulsions were increased. This most likely reflects a reduced compensatory extrusion of sodium from the cells to the haemolymph, probably caused by a reduced production of ATP.

The accumulation of sodium in the intracellular compartments probably leads to an osmotic swelling of cells. Cell swelling is likely to initiate active volume regulation by the extrusion or deamination of FAAs. Reduced total concentrations of FAAs in these amphipods might thus be ascribed to volume regulation of the swollen cells, by extrusion of the FAAs, and a reduced sodium cotransport of FAAs from the haemolymph back to the intracellular compartments.

Combating oil spills and cleaning oil from the tidal zone have recently, during the Exxon Valdes incident, proved to be a difficult and an expensive task in Arctic areas. The present results from the mortality studies might indicate that some dispersants could be used in certain situations to combat oil spills in such areas, probably without increasing the mortality rates of exposed marine organisms. However, the results also indicate that the dispersant should be carefully chosen to avoid additional toxic effects on organisms from the chemical employed. Before any general conclusions regarding the use of dispersants in combating oil spills in Arctic waters can be drawn, experiments must be performed on a variety of other marine species from these areas.

Studies of the physiological effects of sublethal concentrations of crude oil and dispersants indicate that the mode of action of oil and chemicals is complex. The finding of sublethal effects indicates that the physiology of the organisms is affected in manners that do not result in acute mortality. However, the effects evidenced on the physiological parameters investigated in this study could probably prove to be lethal in the long run. Studies of the changes in physiological factors may thus be useful in the attempt to establish tolerance limits of various pollutants, provided that the relationship between the toxicity of the substance and changes in the physiological factors caused by the substance is known. Such studies should preferably be included in routine toxicity testing.

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