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Alterations in the energy budget of Arctic benthic species exposed to oil-related compounds

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Abstract

We studied cellular energy allocation (CEA) in three Arctic benthic species (*Gammarus setosus* (Amphipoda), *Onisimus litoralis* (Amphipoda), and *Liocyma fluctuosa* (Bivalvia)) exposed to oil-related compounds. The CEA biomarker measures the energy budget of organisms by biochemically assessing changes in energy available (carbohydrates, protein and lipid content) and the integrated energy consumption (electron transport system activity (ETS) as the cellular aspect of respiration). Energy budget was measured in organisms subjected to water-accommodated fraction (WAF) of crude oil and drill cuttings (DC) to evaluate whether these compounds affect the energy metabolism of the test species. We observed significantly lower CEA values and higher ETS activity in *G. setosus* subjected to WAF treatment compared to controls ($p \leq 0.03$). Higher CEA value and lower cellular respiration were observed in *O. litoralis* exposed to DC compared to controls ($p = 0.02$). No difference in the energy budget of *L. fluctuosa* was observed between the treatments ($p \geq 0.19$). Different responses to oil-related compounds between the three test species are likely the result of differences in feeding and burrowing behavior and species-specific sensitivity to petroleum-related compounds.

Keywords: Cellular energy allocation (CEA); Arctic; Petroleum; PAH; Biomarkers; Benthos

1. Introduction

Petroleum industry activities are increasing in the Arctic Barents Sea. However, there is a general lack of information concerning possible effects of oil-related chemicals in Arctic ecosystems (Chapman and Riddle, 2005). It is therefore necessary to assess effects of petroleum-related compounds on Arctic organisms (Peterson et al., 2003).

The relative composition of polyaromatic hydrocarbons (PAHs) are known to vary substantially among different crude oil types (Neff, 2002). These compounds and byproducts may bioaccumulate in organisms, resulting in a disruption of cellular and tissue function (French-McCay, 2004). In marine invertebrates exposed to oil-related compounds, reduced growth and reproduction, increased heart rate, and oxidative damage have been documented in experimental studies (Johnson, 1977; Solé et al., 1996; Camus et al., 2002a,b; Neff, 2002; Stark et al., 2003). PAH contamination is for such reasons a matter of concern also in the marine environment.

Drill cuttings are barium-rich byproducts of drilling activities and are typically deposited on the seabed close to drilling sites. While water-based drill cuttings in common usage today are considered to be the least toxic among the different drill cutting types (Sanders and Tibbetts, 1987), their deposition may affect marine organisms by smothering, and by altering the composition of metals in the sediment (Sanders and Tibbetts, 1987; Breuer et al., 2004).

Marine benthic organisms have been widely used in ecotoxicological studies to assess effects of different compounds,

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including oil-related chemicals (Johnson, 1977; Camus et al., 2003; Depledge, 1984). Benthos is relevant as test species as they have been shown to possess compensatory responses to pollutants and often have a sessile life style. Biomarker responses will therefore give an integrated response of pollutants at a specific site (Blaise et al., 1999; Hoarau et al., 2004; Lecoeur et al., 2004).

Earlier studies have shown that some biomarkers for oil exposure used with temperate species may also be relevant for Arctic organisms (Camus et al., 2002a,b, 2003). Biomarkers related to metabolic cost have been developed for temperate species to evaluate effects of pollutants. Pollutant stress may increase the maintenance requirement and hence result in reduced energy available for growth and reproduction (Calow and Sibly, 1990; Huggett et al., 1992; De Coen and Janssen, 2003a,b). The cellular energy allocation (CEA) biomarker was developed by De Coen and Janssen (1997) to provide measurements of the energy status of organisms (Verslycke and Janssen, 2002; De Coen and Janssen, 2003a,b; Verslycke et al., 2004). Calorimetric measurements of available energy (protein, lipid and carbohydrate content) and energy consumed (electron transport system activity (ETS)) are combined into a value expressing the net energy budget at the cellular level (De Coen and Janssen, 1997). The method has proven to be ecologically relevant as cellular effects have been linked to higher levels of biological organization (De Coen and Janssen, 2003a,b; Smolders et al., 2004). CEA is also considered a sensitive biomarker as changes in CEA have been shown to be an order of magnitude more sensitive in some species than, for example, the scope of growth assay (Verslycke et al., 2003). To our knowledge, no studies have used CEA as a biomarker to study effects of oil, and CEA has not previously been used with Arctic species. In this study, we measured changes in carbohydrate-, protein-, and lipid budgets, ETS activity, cellular respiration and CEA in the Arctic benthic species *Onisimus litoralis*, *Gammarus setosus* and *Liocyma fluctuosa* exposed to low levels of petroleum-related compounds.

2. Material and methods

2.1. Species under study

The amphipods *Gammarus setosus*, *Onisimus litoralis* and the bivalve *Liocyma fluctuosa* were chosen as test species in this study. *G. setosus* is typically found in the benthic subtidal or low intertidal regions, and it is the dominant species on the Svalbard East coast (Węslawski et al., 1999). They often consume animal material that has settled to the sea floor. Due to their primarily scavenging nature, they are identified as detrivores and they serve a crucial role in the diet of a variety of species (Wisenden et al., 1999).

O. litoralis is a key species in Arctic soft sediment littoral zone, constituting up to 95% of total fauna density in some areas (Węslawski et al., 2000). It has a narrow environmental distribution as it is limited to waters less than 5 m in depth. *O. litoralis* has a 2.5-year life-span with a single brood incubated from November to April/May (Węslawski et al., 2000). The amphipod has been characterized as a facultative carnivore (Sainte-Marie et al., 1989; Węslawski, 1990; Legezyńska et al., 2000) and is an important food source for waders (*Calidris* spp.), black guillemots (*Cephus grylle*), eiders (*Somateria* spp.) and terns (*Sterna* spp.) (Bradstreet, 1980; Stempniewicz and Węslawski, 1992).

L. fluctuosa inhabits the littoral and sublittoral zone from 1 to 100 m depth. It is distributed from the northern part of the Sea of Japan to the Arctic Ocean. *L. fluctuosa* is a suspension-feeding bivalve and has been shown to feed on phytoplankton (McMahon et al., 2006). *L. fluctuosa* has a high ability to bioaccumulate xenobiotics due to its filtration activities (Holte et al., 2006). Other aspects of the biology of *L. fluctuosa* are poorly known.

2.2. Study area and animal collection

Organisms were collected on 2–7 July 2004 from Thiisbukta; a small cove close to Ny-Ålesund (78°56′N, 11°56′E), Svalbard. *G. setosus* were hand-picked from the area between Thiisbukta and the shore. *O.litoralis* and *L.fluctuosa* were collected by sieving sediment collected with a shovel. Organisms were transported to the laboratory facilities in Ny-Ålesund and kept in aquaria without sediment for two days prior to the start of experiment.

2.3. Set-up and experimental conditions

A sediment layer of 3 cm was added to each aquarium collected from sampling location and 2 l of seawater (from Ny-Ålesund) was then introduced gently into each aquarium. For each of the three test species, 3 replicate aquaria (with 20 individuals in each aquarium) were prepared for each of the following treatments: water-accommodated fraction (WAF), drill cuttings (DC) and control.

DC treatments were prepared by mixing water-based DC with seawater (1:1 mixing ratio) in a standard blender. 44 ml of slurry was added in small doses to each aquarium and allowed to sink through the water column thereby achieving an even distribution of DC (2 mm) on the sediment–water interface.

The water-accommodated fraction (WAF) was made by adding crude oil on top of seawater according to Singer et al. (2000). 6.5 ml of the WAF solution was added to the aquaria. After the treatments were added and allowed to settle, organisms were randomly distributed to aquariums. Aquariums were constantly aerated throughout the experiment. Temperature and salinity was measured each day and before and after water changes throughout the 21-days experiment. The *in situ* water temperature on the first day of collection in Thiisbukta was 6 °C. Average temperature in the aquariums was 4 °C and average salinity in the aquaria was 30 psu. Water samples from each aquarium were taken every third day, before and after water changes. Water samples were also taken from the stock solution every third day.

2.4. Sampling

Animals from the aquaria were sacrificed after 21 days. Sediment samples were taken for each aquarium (surface, mid and bottom layer) after carefully checking for organisms. The remaining sediment was sieved to recover all animals. Animal length was measured after removal from sediment and prior to transferral to a cryogenic vial containing 200 μL Hendrickson buffer (De Coen, 1999). Animals were maintained in liquid nitrogen. Two weeks before analyses, animals were transferred to a freezer (−80 °C). For each species, 70–100 animals were also collected as reference material (day 0). These were stored in liquid nitrogen immediately after collection.

2.5. Sample preparation

Each individual was thawed and immediately homogenized in ice-cold homogenization buffer (De Coen, 1999). The total volumes were then adjusted with homogenization buffer to a total volume of 1 ml. An aliquot of 200 μl homogenate was taken for lipid analysis and 300 μl for protein and glycogen analysis. Samples were stored in liquid nitrogen and in 80 °C until analysis. The ETS measurements were done immediately following homogenization. All samples were analyzed randomly and in triplicates.

2.6. Energy available (Ea)

Protein, carbohydrate and lipid were analyzed according to De Coen (1999). Briefly, protein content was measured by the method described by Lowry et al. (1951) using bovine γ globulin as a standard.

Carbohydrate content was analyzed with anthrone reagent using spectrophotometry at a wavelength of 490 nm (De Coen, 1999). Carbohydrate concentrations were calculated by means of a standard curve of glycogen.

Lipids were extracted following the method of Bligh and Dyer (1959) and lipid concentrations were calculated by reference to standards of tripalmitine in chloroform. Lipid content was quantified photometrically at 340 nm.

2.7. Energy consumed (Ec)

ETS activity was measured according to De Coen and Janssen (1997). Briefly, after homogenization, amphipod and bivalve tissue were centrifuged for 10 min (3000 × g, 4 °C). Aliquots of the homogenate were diluted and 50 μl buffered substrate solution was pipetted out in microwells. 50 μl homogenate and 50 μl of the NADH/NADPH solution were added. The reaction was started by adding 100 μl 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5 phenyl tetrazolium chloride (INT; 0.8 mM) and the absorbance was measured kinetically at 490 nm at 20 °C for 10 min. To calculate EC, ETS activity was transformed into energetic equivalents using an oxyenthalpic equivalent of 480 kJ/mol O_2. Both the ETS and Ec values for each species are reported in this study.

2.8. Calculations

Standard curves for carbohydrates were prepared by using increasing concentrations of glycogen. Increasing concentrations of tripalmitine in chloroform was used for lipids, and for proteins, standard curve with increasing concentration of bovine γ globulin were prepared (De Coen, 1999). The absorption values from the lipid, protein and carbohydrate analyses were converted into concentrations of the respective energy components based on the standard curves. Total concentration of lipid, protein and carbohydrate per organisms were calculated and converted into energy units (39.6 kJ/g lipids, 17.5 kJ/g carbohydrates and 34 kJ/g protein) (Gnaiger, 1983). The energy available (Ea) was calculated for day 0 and day 21; then, ΔEa was calculated by integrating the change in energy reserve components over the 21 day experimental exposure time. Energy consumed (Ec) was calculated at day 0 and 21 from the ETS values on the basis of the Lambert–Beer Law and from oxygen consumption rates (De Coen, 1999), and then Δ in Ec over a 21 day period was calculated. Oxygen consumption values were converted into energy units using the specific oxyenthalpic equivalents for proteins, lipids and carbohydrates (Gnaiger, 1983). The cellular energy allocation (CEA) which represents the total energy budget was calculated according to De Coen and Janssen (1997):

$$\mathrm{CEA(mJ/g\,org)} = \frac{\int_0^t \mathrm{Ea} \times \mathrm{d}t - \int_0^t \mathrm{Ec} \times \mathrm{d}t}{t}$$

2.9. PAH analyses

PAH concentrations in water were analyzed according to the modified standard US Environmental Protection Agency (EPA) procedure 3510 C. Briefly, internal standards were added to water samples and extracted with dichloromethane. Extracts were concentrated and cleaned on a silica column. Samples were analysed by gas chromatography/mass spectrometry and quantifications were made using internal standards. PAH analyses were performed at UNILAB analyse, Tromsø, Norway, accredited laboratory.

3. Statistical methods

Statistical analyses were performed using Statistica® (Ver. 6.1). Each species was analyzed separately. Levene's test was used to test for normality and homogeneity of variance. When there was homogeneity in variance, a one-way measures analysis of variance (ANOVA) was used to test for differences in CEA among the different treatments. When no homogeneity in variance was found, the nonparametric Kruskal–Wallis ANOVA was used. When a significant treatment effect was found, the unequal N HSD post-hoc test was applied to distinguish differences. To check for aquaria effects the generalized linear model nested ANOVA (nested in treatment) was applied. The significance level chosen for all analyses was $\alpha = 0.05$.

4. Results

4.1. G. setosus

There were no significant differences among the treatments in any of the energy available parameters (carbohydrate, protein and lipid contents and their respective energy fractions or in Ea) (ANOVA: $p \geq 0.26$). However, the energy consumed parameters were affected by WAF. ETS activity and Ec was significantly higher in WAF compared to controls (ANOVA: $p \leq 0.03$). CEA was significantly lower in WAF treatment compared to controls (ANOVA: $p = 0.04$), indicating that the WAF treatment affected the energy budget of *G. setosus*. No significant differences between DC and controls were found (ANOVA: $p \geq 0.24$), indicating that DC did not affect the energy budget of *G. setosus* (Fig. 1A).

4.2. *L. fluctuosa*

Protein contents and the respective energy fractions were higher in WAF compared to DC (ANOVA, $p = 0.02$). There was no significant difference among the treatments in any of the other energy budget parameters (carbohydrate and lipid contents and their respective energy fractions, Ea, ETS activity, Ec, and CEA) (ANOVA and Kruskal–Wallis ANOVA, $p \geq 0.19$). These results indicate that the WAF and DC treatments did not affect the energy budget of *L. fluctuosa* (Fig. 1B).

4.3. *O. litoralis*

There were no significant differences among the treatments in any of the energy available parameters (carbohydrate, protein and lipid contents and their respective energy fractions, nor Ea

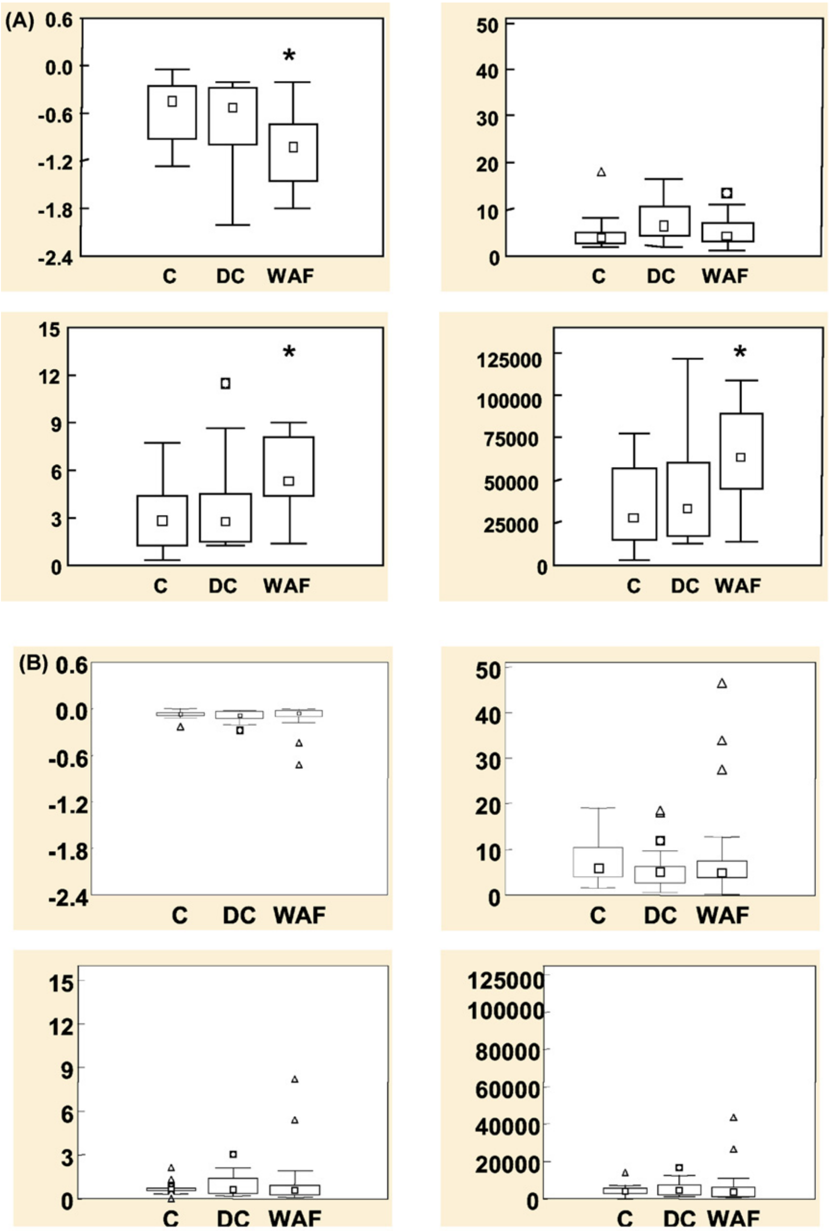

Fig. 1. Cellular energy allocation (CEA), total energy available (Ea (kJ/mm)), electron transport system activity (ETS) (d*A*/d*t*), and total energy consumed (Ec (kJ/mm)) in *Gammarus setosus* (A), *Liocyma fluctuosa* (B) and *Onisimus litoralis* (C) for the following treatments at day 21: drill cuttings (DC), water-accommodated fraction (WAF) and control (C). Median (□), 25 and 75 percentiles (lower and upper bar, respectively), and extreme values (Δ) are shown. (*) indicate significant difference from the control.

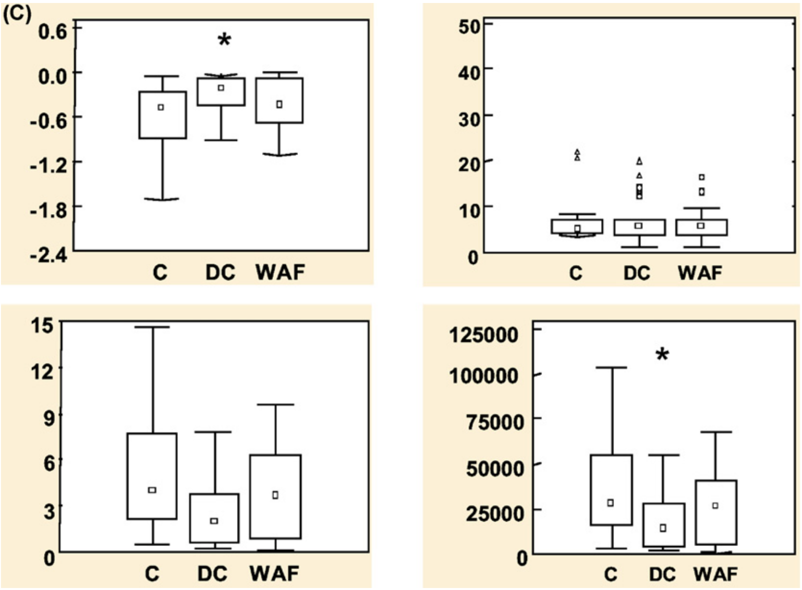

Fig. 1. (*Continued*).

(ANOVA: $p \geq 0.18$) or ETS activity (Kruskal–Wallis ANOVA: $p = 0.08$). However, Ec was lower in DC compared to controls (ANOVA: $p = 0.022$). The CEA value was significantly higher in DC treatment compared to controls (ANOVA: $p = 0.02$). The result indicates that the energy metabolism of *O. litoralis* was altered by DC treatment (Fig. 1C).

4.3.1. Other variables

In all the three test species, CEA values were lower at day 21 (controls) compared to the reference group (day 0) ($p \leq 0.00001$). In *G. setosus, O. litoralis, L. fluctuosa* the average CEA values at day 0 (reference group) and at day 21 (control) were 4.8 and −0.5, 6.0 and −0.5, 7.4 and −0.06, respectively. This finding indicates that all three species consumed more energy than they gained during the course of the experiment.

There was no correlation between CEA and length of animals for any of the test species ($p \geq 0.21$) (*L. fluctuosa*: $r^2 = -0.078$, $p = 0.58$. *G. setosus*: $r^2 = -0.197$, $p = 0.21$. *O. litoralis*: $r^2 = -0.797$, $p = 0.56$), indicating that CEA value was not affected by animal size. Similarly, for all test species, there was no difference in animal length in the different treatments ($p \geq 0.655$). Hence, the size distributions of the animals selected for the different treatments were similar.

Aquarium number did not affect the CEA value in any of the three test species (nested ANOVA: $p \geq 0.39$). Using Kruskal–Wallis for *O. litoralis* and *L. fluctuosa*, no effect of aquaria was found ($p \geq 0.16$).

4.3.2. PAH analyses

Naphthalene, naphthalene C1, naphthalene C2 and naphthalene C3 were the dominating PAH congeners found in water samples at day 0 (representing 87% of the PAHs) (Table 1).

Table 1
PAH concentrations (μg/l) (28 different PAH congeners, sum naphthalene–phenantrene–dibenzothiophene (NPD) and sum 16 EPA) in aquaria at day 0 (dissolved concentrations)

PAH	μg/l
Naphtalene	8.40
C1-naphtalene	75.43
C2-naphtalene	67.42
C3-naphtalene	20.41
Phenantrene	3.19
Antracene	0.20
C1-phenantrene/antracene	3.75
C2-phenantrene/antracene	2.20
C3-phenantrene/antracene	0.85
Dibenzotiophene	0.88
C1-dibenzotiophene	2.22
C2-dibenzotiophene	2.86
C3-dibenzotiophene	2.33
Acenaphtylene	0.26
Acenaphtene	0.26
Fluorene	2.11
Fluorantene	0.04
Pyrene	0.03
Benzo[a]antracene	0.01
Krysene	0.03
Benzo[b]fluorantene	BD
Benzo[k]fluorantene	BD
Benzo[e]pyrene	0.003
Benzo[a]pyrene	0.001
Perylene	BD
Indeno[1,2,3-cd]pyrene	BD
Benzo[ghi] perylene	BD
Dibenzo[a,h]antracene	BD
SUM NPD	190.13
SUM 16 EPA	14.53

BD = below detection limit.

5. Discussion and conclusion

We show that CEA is a potential additional biomarker to study effects of oil-related compounds in some Arctic benthic organisms, and our results are the first to demonstrate that WAF and DC exposure may affect the energy budget of Arctic benthic species. A higher Ec and ETS activity and a lower CEA value in WAF exposed organisms compared to controls was found in *G. setosus*. In *O. litoralis*, a lower Ec and higher CEA value was observed in DC compared to controls. WAF and DC did not affect the energy budget in *L. fluctuosa*. The reference organisms (day 0) all had positive CEA values, and were probably able to allocate energy to their reserves. However, across treatments a negative CEA value was observed in all test organisms, indicating that animals used more energy (respiration) than they were able to allocate to their reserves. Since the CEA values of control animals also were negative, the organisms were probably stressed by the experimental conditions, including food shortage, because animals were not fed during the experimental period. The reasons why the treatment caused additional stress in *G. setosus* and *O. litoralis* and not *L. fluctuosa* may be related to differences in susceptibility to oil-related compounds among the test species, differences in burrowing activity and dietary preferences.

A lower CEA value in *G. setosus* exposed to WAF compared to controls shows that WAF caused sub lethal stress in *G. setosus* that induced compensatory changes in its energy metabolism. A decline in CEA indicates either a reduction of available energy or higher energy expenditure. In the present study, lower CEA value of *G. setosus* in WAF was caused by higher energy expenditure and not by reduction of energy available since only the energy consumed parameters (Ec) were affected by WAF. This suggests that WAF enhanced catabolism in *G. setosus*. Ec, which corresponds to cellular respiration, and ETS activity are therefore the most sensitive fractions of the energy budget of *G.setosus* following WAF exposure. This is in accordance with a study from Aunaas et al. (1991) who reported an increased respiration rate (whole animal) in *Gammarus oceanicus* from Svalbard fjords exposed to WAF. WAF exposure was found to increase membrane permeability to water and ions, leading to an increased influx of water and sodium from the medium to the organisms. The increased respiratory rates could be due to the compensatory extrusion of sodium. In addition, Hatlen and Camus (pers. comm.) have found that respiration was the most sensitive parameters in the ice amphipod *Gammarus wilkitzkii* exposed for one month to the water-soluble fraction compared to parameters related to oxidative stress metabolism. Growth, reproduction and maintenance metabolism are the most important fractions of the energy expenditure of organisms (Calow and Sibly, 1990; De Coen and Janssen, 2003a,b). A lower CEA value in *G. setosus* exposed to WAF suggests that less energy was available for growth and reproduction in this experiment.

The observed response to WAF treatment in *G. setosus* was probably caused by low molecular weight, water-soluble compounds of crude oil. Larger molecules associated with long-term effects are not water-soluble and are probably retained in the oil phase of the WAF (Short et al., 2003). Naphtalene, benzene, toluene, ethylbenzene, and xylene (BTEX) are assumed to account for most of the toxicity of the low molecular weight monocyclic aromatic hydrocarbons. These compounds have been shown to induce narcosis in marine organisms (Peterson, 1994). Compensatory mechanisms for narcosis, like increased heart rate and increased respiration have previously been documented in marine organisms (Depledge, 1984; Aunaas et al., 1991; Camus et al., 2002a,b). It is therefore conceivable that the increased cellular respiration observed in *G. setosus* in our study was partly caused by narcotic effects of the low molecular weight compounds of the WAF, especially the naphthalenes that dominated the PAH congener pattern.

The energy budgets of *O. litoralis* and *L. fluctuosa* did not appear to be affected by WAF treatment. Earlier studies have shown that WAF of crude oil is not very toxic to amphipods, compared to direct exposure to crude oil (Gulec et al., 1997). Reasons for the response to WAF in *G. setosus* and not in *O. litoralis* and *L. fluctuosa* may be related to differences in susceptibility to PAHs, since response to PAH exposure varies greatly among phyla (Livingstone, 1994). Gesteira and Dauvin (2000) demonstrated that following an acute oil spill, recovery times of amphipods were greater than for other benthic species because they are brooders (no pelagic larvae) and therefore have a slow population expansion. In contrast, bivalves accumulate PAHs to higher concentrations compared to other organisms as observed in *Mya truncata* exposed to oil contaminated sediment (Camus et al., 2003). Bivalves are also known to be able to close their shells and protect themselves from toxic substances (Tran et al., 2004). The Ec, ETS and CEA values of *L. fluctuosa* indicate that it possessed a low metabolic level, probably due to closed shells. The energy budget of *L. fluctuosa* may have been modified at the beginning of the oil exposure (first days of exposure), and then the bivalve may have closed its shells and possibly reduced its metabolism to avoid exposure to toxic compounds. This could explain why the WAF and the DC did not affect the energy budget of this organism.

In another study using the same treatment as herein, a bivalve-dominated temperate community showed no response to crude oil exposure, while increased respiration was documented in a polychaete-dominated Arctic community (Olsen et al., in press). These results suggest that bivalves are less affected by a short-term exposure to oil-related compounds compared to other species, and this may also explain why we observed a response in the amphipod *G. setosus* and no response in the bivalve *L. fluctuosa*.

Differences in dietary preferences may also explain why *G. setosus* responded to WAF treatment while *O. litoralis* did not. Wheelock et al. (2002) showed that poor nutritional state of aquatic organisms resulted in increased toxicity to WAF. Since *Onisimus* spp. is a carnivore necrophage, and digests meals very slowly, they can tolerate long periods of starvation. In contrast, *G. setosus* is classified as generalist or detrivore (Legeżyńska et al., 2000). It is therefore possible that *O. litoralis* tolerated food shortage better than *G. setosus* (animals were not fed during the 21-day experiment). *O. litoralis* may therefore have been able to cope better with the additional stress caused by WAF exposure compared to *G. setosus*.

Compounds entering the water-phase of a WAF are water-soluble. Therefore, when WAF was added to the aquaria, most of the compounds would remain in the water-phase, and would not become associated with the sediment. During the experiment, *G. setosus* was observed in the water. *G. setosus* may therefore have been exposed to PAHs to a greater extent compared to *O. litoralis* and *L. fluctuosa* as the latter species were primarily located buried in the sediment. Burrowing behavior may therefore explain a response in *G. setosus* and a lack of response in *O. litoralis* and *L. fluctuosa* to the WAF treatment.

Sampling site may also be an explanatory factor for different responses to WAF among the species. *O. litoralis* and *L. fluctuosa* were collected from sediment from Thiisbukta, while *G. setosus* was collected further out, closer to the shore. Thiisbukta is characterized by frequently low salinity and large temperature changes due to glacial runoff. Animals living there may therefore generally be more tolerant to different stressors compared to animals inhabiting areas exhibiting more stable environmental conditions.

CEA measurements were done at only one time point (day 21), reflecting the energy budget at this particular moment. However, CEA can respond at early time points of experiments and the response may be transient (Verslycke and Janssen, 2002; De Coen and Janssen, 2003a,b; Verslycke et al., 2004). Because of the limited resources available in the Arctic (animal availability, laboratory space and logisitics) no additional sampling points could be done in our study; therefore, the lack of responses measured in *L. fluctuosa* can be related to the late sampling time point.

O. litoralis was the only species in this study that appeared to be affected by DC. Explanations for lower Ec value (thus a higher CEA) may be related to the effect of smothering. The DC layer on top of the sediment may have decreased oxygen diffusion from the water into the sediment. Less oxygen in the sediment of DC aquariums may have resulted in a decreasing activity of *O. litoralis*, and hence a lower respiration, resulting in a higher CEA value. For *G. setosus*, the lack of response to DC was probably related to the fact that *G. setosus* did not bury in the sediment and was therefore not subjected to low oxygen concentrations. *L. fluctuosa* in control aquaria stayed in the sediment most of the time during the experiment. However, *L. fluctuosa* in DC remained on top of the DC layer with open valves during the first days of the experiment. Such avoidance behaviour may have been beneficial to the animal since no CEA changes were measured.

In all cases, much higher values of Ec compared to Ea were measured. This can be explained by several factors: (1) it is assumed that CEA activity is an overestimation of the actual respiration (e.g. Båmstedt, 1980; Skjoldal et al., 1984; Verslycke and Janssen, 2002) because the method measures the maximum ETS activity under saturated substrate concentrations; and (2) Ec values are directly related to enzyme activity measurement which is a snapshot view of energy consumption but not an integration over time of what the cell has actually consumed.

In parts of Svalbard waters, the benthic amphipods *O. litoralis* and *G. setosus* dominate, and they provide a link between primary production, detritus and higher trophic levels (Węslawski et al., 1999, 2000; Hop et al., 2006). Our results provide preliminary evidence that benthic species' energy budgets are affected differently by oil-related compounds. In this first analysis of CEA in Arctic benthic organisms WAF affected the energy budget in *G. setosus*, while DC affected the energy budget in *O. litoralis*. *L. fluctuosa* was not affected by any of the treatments. However, more time points are certainly needed to definitively link the responses of energy budget parameters to both petroleum hydrocarbons and drill cuttings.

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References

Aunaas, T., Olsen, A., Zachariassen, K.E., 1991. The effects of oil and dispersants on the amphipod Gammarus oceanicus from Arctic waters. In: Sakshaug, E., Hopkins, C.C.E., Øritsland, N.A. (Eds.), Proceedings of the Pro Mare Symposium on Polar Marine Ecology. Trondheim, 12–16 May 1990. Polar Research 10 (2), pp. 619–639.

Båmstedt, U., 1980. ETS activity as an estimator of respiratory rate of zooplankton populations. The significance of variations in environmental factors. J. Exp. Mar. Biol. Ecol. 42, 267–283.

Blaise, C., Gagné, F., Pellerin, J., Hansen, P.D., 1999. Determination of vitellogenin-like properties in *Mya arenia* hemolymph (Saguenay, Fjord, Canada): a potential biomarker for endocrine disruption. Environ. Toxicol. 14, 455–465.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bradstreet, M.S.W., 1980. Thick billed murres and black guillemots in the Barrow Strait area, NWT during spring. Diets and food availability along ice edges. Can. J. Zool. 58, 2120–2140.

Breuer, E., Stevenson, A.G., Howe, J.A., Carroll, J., Shimmield, G.B., 2004. Drill cuttings accumulations in the Northern and Central North Sea: a review of environmental interactions and chemical fate. Mar. Pollut. Bull. 48 (1–2), 12–25.

Calow, P., Sibly, R., 1990. A physiological basis of population process: ecotoxicological implications? Funct. Ecol. 4, 283–288.

Camus, L., Jones, M.B., Børseth, J.F., Regoil, F., Depledge, M.H., 2002a. Heart rate, respiration and sum oxyradical scavenging capacity of the Arctic spider crab *Hyas araneus*, following exposure to polycyclic aromatic compounds via sediment injection. Aquat. Toxicol. 62 (1–2), 1–13.

Camus, L., Jones, M.B., Børseth, J.F., Grosvik, B.E., Regoil, F., Depledge, M.H., 2002b. Total oxyradical scavenging capacity and cell membrane stability of haemocytes of the Arctic scallop, *Chlamys islandicus*, following benzo(a)pyrene exposure. Mar. Environ. Res. 54 (3–5), 425–430.

Camus, L., Birkely, S.R., Jones, M.B., Børseth, J.F., Grosvik, B.F., Gulliksen, B., Lonne, O.J., Regoli, F., Depledge, M.H., 2003. Biomarker responses and PAH uptake in Mya truncata following exposure to oil-contaminated sediment in an Arctic fjord (Svalbard). Sci. Total Environ. 308 (1–3), 221–234.

Chapman, P.M., Riddle, M.J., 2005. Toxic effects of contaminants in polar marine environments. Environ. Sci. Technol. 39 (9), 200A–207A.

De Coen, W.M., Janssen, C.R., 1997. The use of biomarkers in *Daphnia magna* toxicity testing: IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed Daphnia populations. J. Aquat. Ecosyst. Stress Recovery 6, 43–55.

De Coen, W., 1999. Energy metabolism, DNA damage and population dynamics of the waterflea *Daphnia magna* Straus under toxic stress. Ph.D. thesis. Department of Biochemistry and Toxicology, Gent.

De Coen, W.M., Janssen, C.R., 2003a. A multivariate biomarker-based model predicating population-level responses of *Daphnia magna*. Environ. Toxicol. Chem. 22 (9), 2195–2201.

De Coen, W.M., Janssen, C.R., 2003b. The missing biomarker link: relationship between effects on the cellular energy allocation biomarker of toxicant-stressed *Daphnia magna* and corresponding population characteristics. Environ. Toxicol. Chem. 22 (7), 1632–1641.

Depledge, M.H., 1984. Changes in cardiac activity, oxygen uptake and perfusion indices in *Carcinus maenas* (L.) exposed to crude oil and dispersant. Comp. Biochem. Physiol. C Comp. Pharmacol. 78 (2), 461–466.

French-McCay, D.P., 2004. Oil spill impact modeling: development and validation. Environ. Toxicol. Chem. 23 (10), 2441–2456.

Gesteira, J.L.G., Dauvin, J.C., 2000. Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. Mar. Pollut. Bull. 40 (11), 1017–1027.

Gnaiger, E., 1983. Appendix C. Calculation of energetic and biochemical equivalents of respiration oxygen consumption. In: Ga, Forstner (Ed.), Polarographic Oxygen Sensores. Springer Verlag, Berlin Heidelberg, pp. 337–345.

Gulec, I., Lenrad, B., Holdway, D.A., 1997. Oil and dispersed oil toxicity to amphipods and snails. Spill Sci. Technol. Bull. 4 (1), 1–6.

Hoarau, P., Garello, G., Gnassia-Barelli, M., Romeo, M., Girad, J.P., 2004. Effect of three xenobiotic compounds on glutathione S-transferase in the clam *Ruditapes decussates*. Aquat. Toxicol. 68 (1), 87–94.

Holte, B., Dahle, S., Gulliksen, B., Næs, K., 2006. Some macrofaunal effects of local pollution and glacier-induced sedimentation, with indicative chemical analyses, in the sediments of two Arctic fjords. Polar Biol. 16 (8), 549–557.

Hop, H., Falk-Petersen, S., Svendsen, H., Kwasniewski, S., Pavlov, V., Pavlova, O., Søreide, J.E., 2006. Physical and biological characteristics of the pelagic system across Fram Strait to Kongsfjorden. Prog. Oceanography 71 (2–4), 182–231.

Huggett, R.J., Kimerle, R.A., Mehrle Jr., P.M., Bergman, H.L., 1992. Biomarkers: Biomchemical, Physiological, and Histological Markers of Anthropogenic stress. Lewis, Boca Raton, FL.

Johnson, F.G., 1977. Sub lethal biological effects of petroleum hydrocarbon exposures: bacteria, algae and invertebrates. In: Malins, D.C. (Ed.), Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms. Biological Effects, 2. Academic Press, N.Y., pp. 271–318.

Lecoeur, S., Videmann, B., Berny, P., 2004. Evaluation of metallothionein as a biomarker of single and combined Cd/Cu exposure in *Dreissena polymorpha*. Environ. Res. 94, 184–191.

Legezyńska, J., Węslawski, J.M., Presler, P., 2000. Benthic scavengers collected by baited traps in the high Arctic. Polar Biol. 23, 539–544.

Livingstone, D.R., 1994. Recent developments in marine invertebrate organic xenobiotic metabolism. Toxicol. Ecotoxicol. News 1, 88–95.

Lowry, O., Rosebrough, N., Farr, A., Randall, R., 1951. Protein measurement with the Folin Phenol Reagent. J. Boil. Chem. 193, 265–275.

McMahon, K.W., Ambrose Jr., W.G., Johnson, B.J., Sun, M.Y., Lopez, G.R., Clough, L.M., Carroll, M.L., 2006. Benthic community response to ice algae and phytoplankton in Ny Ålesund, Svalbard. Mar. Ecol. Prog. Series 310, 1–14.

Neff, J.M. (Ed.), 2002. Bioaccumulation in Marine Organisms-Effect of Contaminants from oil Well Produced Water. Batelle, Coastal Resources and Environmental Management, Duxbury, Massachusetts 02332, USA pp. 241–313.

Olsen, G.H., Carroll M.L., Renaud P.E., Ambrose W.G. Jr, Olssøn R., Carroll J.L. Benthic community response to petroleum-associated components in Arctic versus temperate marine sediments. Mar. Biol. (in press).

Peterson, C.H., Rice, S.D., Short, J.W., Esler, D., Bodkin, J.L., Ballachey, B.E., Irons, D.B., 2003. Long-term ecosystem response to the *Exxon Valdez* oil spill. Science 302, 2082–2086.

Peterson, D.R., 1994. Calculating the aquatic toxicity of hydrocarbon mixture. Chemosphere 29, 2493–2506.

Sainte-Marie, B., Percy, J.A., Shea, J.R., 1989. A comparison of meal size and feeding rate of the lysianassid amphipods *Anonyx nugax*, *Onisimus* (=*Pseudalibrotus*) *litoralis and Orchomenella pingus*. Mar. Biol. 102, 361–368.

Sanders, P.F., Tibbetts, P.J.C., 1987. Effects of discarded drill muds on microbial populations. Phil. Trans. R. Soc. Lond. B Biol. Sci. 316 (1181), 567–585.

Short, J.W., Rice, S.D., Heintz, R.A., Carls, M.G., Moles, A., 2003. Long-term effects of crude oil on developing fish: lessons from the *Exxon Valdez* oil spill. Energy Sources 25, 509–517.

Singer, M.M., Aurand, D., Bragin, G.E., Clark, J.R., Coelho, G.M., Sowby, M.L., Tjeerdema, R.S., 2000. Standardization of the preparation and quantification of water-accomodated fractions of petroleum for toxicity testing. Mar. Pollut. Bull. 40 (11), 1007–1016.

Skjoldal, H.R., Båmstedt, U., Klinken, J., Laing, A., 1984. Changes with time after capture in the metabolic activity of the carnivorous copepod *Eucheta norvegica* Boeck. J. Exp. Mar. Boil. Ecol. 83 (3), 195–210.

Solé, M., Porte, C., Biosca, X., Mitchelmore, C.L., Chipman, J.K., Livingstone, D.R., Albaigés, J., 1996. Effects of the "Aegean Sea" oil spill on biotransformation enzymes, oxidative stress and DNA-adducts in digestive gland of the mussel (*Mytilus edulis* L.). Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 113 (2), 257–265.

Smolders, R., Bervoets, L., De Coen, W., Blust, R., 2004. Cellular energy allocation in zebra mussel exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. Environ. Pollut. 129, 99–112.

Stark, J.S., Snape, I., Riddle, M.J., 2003. The effect of petroleum hydrocarbon and heavy metal contamination of marine sediments on recruitment of Antarctic soft-sediment assemblages: a field experimental investigation. J. Mar. Biol. Ecol. 283 (1–2), 21–50.

Stempniewicz, L., Węslawski, J.M., 1992. Outline of trophic relationships in Hornsund SW Spitsbergen. In: Opaliński, K.W., Klekowski, R.Z. (Eds.), Landscape, life world and man in High Arctic. IE PAN, Warszawa, pp. 271–298.

Tran, D., Ciret, P., Ciutat, A., Durrieu, G., Massabuau, J.C., 2004. Estimation of potential and limits of bivalve closure response to detect contaminants: applications to cadmium. Environ. Toxicol. Chem. 22, 914–920.

Verslycke, T., Janssen, C.R., 2002. Effects of changing abiotic environment on the energy metabolism in the estuarine mysid shrimp *Neomysis integer* (Crustacea: Mysidacea). J. Exp. Mar. Biol. Ecol. 279, 61–72.

Verslycke, T., Vercauteren, J., Devos, C., Moens, L., Sandra, P., Janssen, C.R., 2003. Cellular energy allocation in the estuarine mysid shrimp *Neomysis integer* (Crustacea: Mysidacea) following tributyltin exposure. J. Exp. Mar. Biol. Ecol. 288, 167–179.

Verslycke, T., Ghekiere, An., Janssen, C.R., 2004. Seasonal and spatial patterns in cellular energy allocation in the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) of the Scheldt estuary (The Netherlands). J. Exp. Mar. Biol. Ecol. 306, 245–267.

Węslawski, J.M., 1990. Distribution and ecology of coastal water amphipoda from Southern Spitsbergen. Polish Archiv fur Hydrobiologie 37, 503–519.

Węslawski, J.M., Stempniewicz, F., Mehlum, F., Kwaíniewski, S., 1999. Summer feeding strategy of little auk (*Alle alle*) from Bjørnøya, Barents Sea. Polar Biol. 21 (3), 129–134.

Węslawski, J.M., Opliński, K., Legeżyńska, J., 2000. Life cycle and production of *Onisimus litoralis* (Crustaces Amphipoda): The key species in the Arctic soft sediment littoral. Polskie Archiwum Hydrobiologii 47, 585–596.

Wheelock, C.E., Baumgartner, T.A., Newman, J.W., Wolfe, M.F., Tjeerdema, R.S., 2002. Effect of nutritional state on Hsp60 levels in the rotifer Brachionus plicatilis following toxicant exposure. Aquat. Toxicol. 61 (1–2), 89–93.

Wisenden, B.D., Cline, A., Sparkes, T.C., 1999. Survival benefit to antipredator behavior in the amphipod *Gammarus minus* (Crustacea: Amphipoda) in Response to injury-released Chemical cues from conspecifics and heterospecifics. Ethology 105, 407–414.