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BioSea II JIP:
Effects of Goliat oil on shrimp larvae
Phase 2: Low concentrations of oil from “Kobbe”

Renée K. Bechmann

REPORT – 2008/126

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BioSea II JIP:

Effects of Goliat oil on shrimp (*Pandalus borealis*) larvae

Phase 2: Low concentrations of oil from "Kobbe"

Author: Renée Katrin Bechmann

Project participants: Solveig Apeland, Thierry Baussant, Anna Ingvarsdottir, Helge Knutsen, Emily Lyng, Marianne Nilsen, Atle Nævdal, Rolf Sundt, Ingrid C. Taban, Anne Helene Tandberg, Stig Westerlund, Kjell Birger Øysæd.

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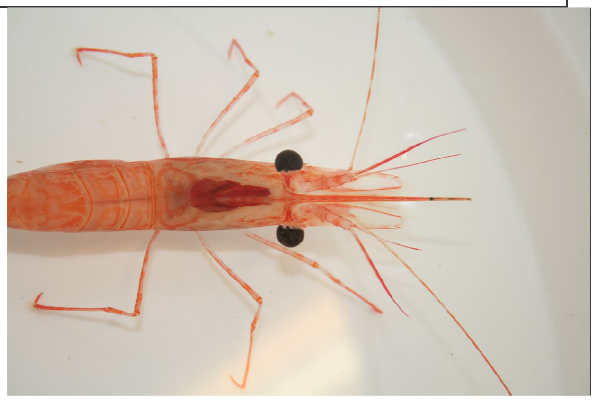

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Summary

Background – Effects of oil on shrimp embryos and larvae

- The results from the 2004 shrimp experiments showed that where embryos were exposed to oil increased mortality of larvae was seen even if the larvae were kept in clean water. LOEC[1] = 0.015 mg/L Goliat oil (0.36 µg/L ∑PAH).
- The results from the 2007 shrimp larvae experiment were that oil caused increased mortality and development time for shrimp larvae exposed to 0.015 mg/L Goliat oil (0.26 µgL ∑PAH).
- Lower concentrations than 0.015 mg/L oil have never been tested on shrimp larvae.

The BioSea II – Phase 2 Shrimp larvae experiment (spring 2008)

The objective of the present experiment was to repeat the lowest oil concentration tested in the 2007 experiment (0.015 mg/L), using a different batch of Goliat oil. To test a concentration that was as low as possible, but that still gave significantly higher PAH concentrations than the control, a 0.010 mg/L concentration of oil was also tested.

Toxicity of oil depends on the chemical composition and physical characteristics of the oil. The oil used in 2007, and in BioSea I, was from Goliat/Real. The batch of Goliat oil used in 2008 was from Kobbe. The concentration of PAH compounds were lower in the Goliat/Kobbe than in the Goliat/Real, and the composition of PAHs and alkanes was also different in the two batches. The measured total concentration of PAH in the exposure aquaria in 2008 was 0.07 µg/L ∑PAH in the 0.010 mg/L oil exposure and 0.12 µg/L ∑PAH in the 0.015 mg/L oil exposure.

Newly hatched larvae were exposed to 0.010 mg/L and 0.015 mg/L dispersed Goliat/Kobbe oil for 35 days, starting less than 24 hours after hatching. The selected endpoints were mortality, development time and growth.

Mortality:

- The mortality in the control was very low. After 5 weeks exposure the mean accumulated mortality was only 8 %.
- The mortalities in the two oil exposed groups were 2-3 % higher than in the control after 35 days exposure. There was, however, no statistical difference between control and exposed groups.

Growth:

- The growth (weight) of shrimp larvae was not affected by 35 days exposure to 0.010 mg/L or 0.015 mg/L Goliat/Kobbe oil.

1 LOEC = Lowest Observed Effect Concentration

Development time:

- Stage II – stage III: The development time from stage II to stage III was, on average, 1 day faster for larvae exposed to 0.010 mg/L Goliat/Kobbe oil than for control larvae. No effect on development time was detected for larvae exposed to 0.015 mg/L Goliat/Kobbe oil.
- Stage III – stage IV: The percentage of larvae that developed to stage IV was not affected by 35 days exposure to 0.010 mg/L or 0.015 mg/L Goliat/Kobbe oil.

There was no statistically significant difference in survival or growth for any treatment. The small increase in development time observed from stage II to stage III for larvae exposed to 0.010 mg/L Goliat/Kobbe oil was no longer present at the end of the experiment when the larvae had developed to stage IV.

Hence we conclude that 0.015 mg/L (0.12 µg/L PAH) is the 35 day NOEC[2] for the Goliat/Kobbe oil.

The lower concentration of PAH and the different chemical composition of the Goliat/Kobbe oil compared to the Goliat/Real oil tested in 2007 are the most likely explanations for the difference in effect of the 0.015 mg/L oil treatment in the two experiments.

2 NOEC: No Observed Effect Concentration. Since no higher concentrations than 0.015 mg/L oil has been tested using the Goliat/Kobbe batch of oil it is not possible to give a LOEC for this batch of oil, and we do not know if NOEC for this oil is higher than 0.015 mg/L.

CONTENTS

1 Preface

The project was financed by ENI Norge AS and Total Norge AS through the BioSea II JIP.

Planning: Renée K. Bechmann and Ingrid C. Taban

Rigging up exposure system: Helge Knutsen, Marianne Nilsen, Anna Ingvarsdottir and Renée K. Bechmann

Maintenance of the experiment, checking survival and determining stage: Anne Helene Tandberg, Marianne Nilsen, Solveig Apeland, Anna Ingvarsdottir, Emily Lyng and Renée K. Bechmann

Fishing adult shrimps: Rolf Sundt

PAH and THC analysis: Atle Nævdal and Kjell Birger Øysæd

CFS maintenance: Thierry Baussant and Rolf Sundt

Coulter counter analysis: Stig Westerlund

Reporting: Renée K. Bechmann

Program manager: Steinar Sanni

QA (report): Marianne Nilsen, Anna Ingvarsdottir, Atle Nævdal and Steinar Sanni

All the background data are given in appendix 1-14 and in a separate Excel file.

Shrimp and wolffish in the aquarium. Photo: A.H. Tandberg

2 INTRODUCTION

The northern shrimp (*Pandalus borealis*) is a key invertebrate species in the Barents Sea. Background information about the selected test species and the relevance of using shrimp larvae in the BioSea JIPs experiments is described in the Introduction of last years report (Taban *et al.,* 2007).

The objective of the present experiment was to study biological effects in shrimp larvae exposed to following nominal concentrations of Goliat oil: 0.010 and 0.015 mg/L oil. The purpose was to determine a NOEC for the oil used.

We have run an experiment where the lowest concentration (0.015 mg/L of oil) tested in the 2007 experiment was repeated using a different batch of Goliat oil. The oil used in 2007, and in BioSea I, was from "Real", and the batch of Goliat oil used in 2008 was from "Kobbe". In addition, an even lower concentration of oil (0.010 mg/L) was included in 2008 to test a concentration that was as low as possible, but that still gave significantly higher PAH concentrations than the control.

The Goliat oil used in the present shrimp larvae experiment was different from the Goliat oil used in BioSea I and Biosea II (phase 1). The toxicity of oil depends on the chemical composition and physical characteristics of the oil; hence we could not assume to find the same effects for the new batch of Goliat oil (Kobbe) as for the oil tested in 2007 (Goliat/Real). This is especially important when the aim is to test concentrations close to the expected NOEC. If the new batch of oil was more toxic than the oil tested in 2007 it would be possible that effects could be found in both the selected concentrations. If the new oil was less toxic than the 2007 oil it could be possible that no effect would be seen in any of the concentrations. In addition, one also has to anticipate variability in the general condition of offspring in different years.

The results from the 2008 experiment can not be used to determine a NOEC for the 2007 oil. The LOEC for the 2007 oil is 0.015 mg/L Goliat/Real oil. Table 1 show results from the 2007 shrimp larvae experiment and 3 possible scenarios for 2008:

Scenario 1: LOEC for oil 2008 = 0.010 mg/L. We can not set a NOEC for oil 2008.

Scenario 2: LOEC for oil 2008 = 0.015 mg/L. NOEC for oil 2008 = 0.010 mg/L.

Scenario 3: NOEC for oil 2008 = 0.015 mg/L. We can not set a LOEC for oil 2008.

Table 1. Results from the 2007 shrimp larvae experiment and 3 possible scenarios for 2008. Red boxes: significant effect. Green boxes: no significant effect. Grey boxes: not tested.

Nominal oil concentrations	Results from 2007	2008 Scenario 1	2008 Scenario 2	2008 Scenario 3
0.010 mg/L Goliat 2008				
0.015 mg/L Goliat 2008				
0.015 mg/L Goliat 2007				
0.060 mg/L Goliat 2007				

3 MATERIALS AND METHODS

3.1 The shrimp (*Pandalus borealis*) adults and larvae

Northern shrimp, *Pandalus borealis*, were collected from a local stock (Rogaland) to minimise the transport period. Sampling was carried out in early February 2008, where adult shrimps were collected by trawling. To minimise damage to the shrimps within the net when brought to the surface, a barrel was securely clamped to the end of the trawl. This was very important since normal trawling often leads to severe mechanical damages to, or loss of, body parts such as antenna and legs, followed by increased mortality at the time of catch as well as later. The trawling period was short, about 15-20 minutes at the bottom, and female specimens bearing developing embryos were immediately transferred to transport tanks. The water used in the transport tanks was cooled (~5°C) seawater from Akvamiljø, which probably also improved survival.

Upon arrival at Akvamiljø, the adult female shrimps were transferred to ~500 L tanks with flow through seawater at 5°C for a minimum of two weeks of acclimatization, prior to transfer to hatching chambers (19 L aquaria with 2 compartments) held at the same temperature. Seawater was taken from 80 m depth (Atlantic water), and passed through a sand filter. Adult shrimps were fed raw fish five times a week, and kept in clean water throughout.

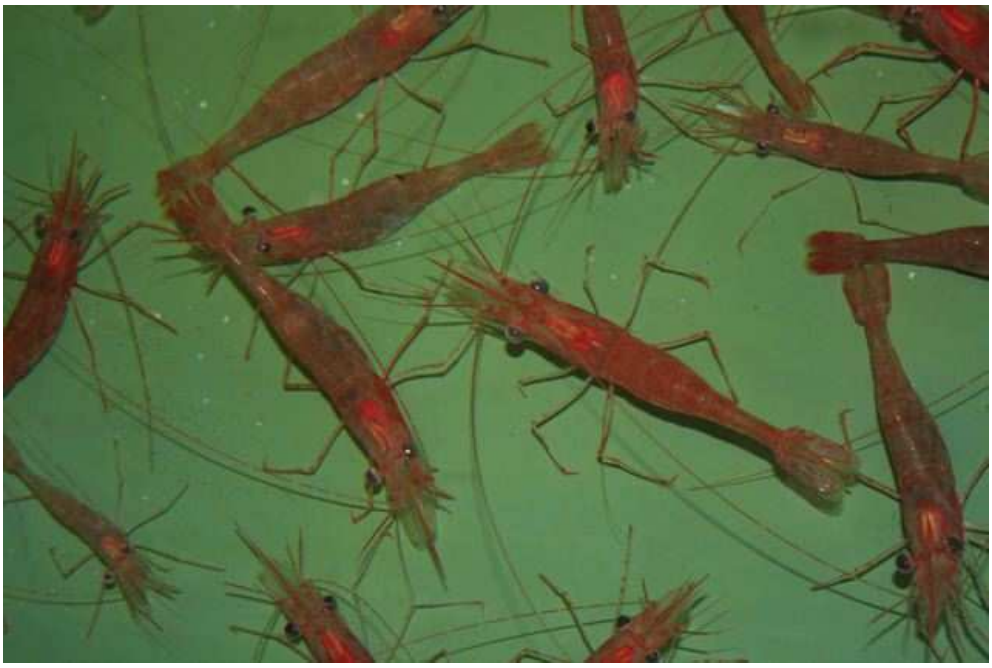

Figure 1. Shrimps in quarantine tank (Photo: R.K. Bechmann).

Female shrimps were placed in separate hatching chambers to collect larvae from each individual and to register the day of hatching of the larvae from each female.

At the time of catch the embryos were blue-green, with the opacity and colour of the eggs decreasing until hatching. Eye spots within the developing embryos were clearly visible in the eggs when adult female shrimps were caught.

The first shrimp larvae hatched during the last week of February, which is similar to the experiment in 2007 but earlier than in the BioSea I when larvae started to hatch in mid March. Approximately 70 adult female shrimps were transferred to the hatching chambers and larvae from 21 of these were used to obtain the 6300 larvae needed for the experiment (same number of females as in the 2007 experiment). The exposure was carried out using only newly hatched shrimp larvae. All the 7 replicates of control and the two oil concentrations were started in the period from 11-14 March 2008.

The larvae were fed once a day with newly hatched *Artemia salina* nauplii. Small *Artemia* cysts (± 430 µm) with high content of highly unsaturated fatty acids (HUFA) were used (AF Specialty *Artemia* cysts, ∑ω3HUFA > 15 mg/g dwt, INVE Aquaculture$_{NV}$). During the first part of the experiment 1.5 g of cysts per day were used to feed all the shrimp larvae and this was increased to 2.5 g as the larvae grew. The cysts were transferred to a 5 L beaker with 3.5 L seawater and 1.5 L tap water, and kept at 30°C with bubbling/oxygenation and light. The nauplii hatched within 24 hours and were siphoned out to avoid including un-hatched cysts. The *Artemia* were then poured through a 40 µm mesh cylinder, rinsed in seawater and concentrated in a 100 ml beaker. Approximately 3 ml of concentrated *Artemia* nauplii were fed to each of the 21 larvae aquaria. The first week after hatching the shrimp larvae were also fed algae (*Thalassiosira weissflogi* Instant algae, Shellfish diet). 100 µl instant algae was diluted with 80 ml seawater and added to each aquarium. Dead *Artemia*, other organic waste and dead shrimp larvae were removed when siphoning out the larvae for stage determination.

Figure 2. Female shrimp with brownish, mature eggs (Photo: A. H. Tandberg).

3.2 Experimental design

Shrimp larvae were exposed to a dispersion of Goliat oil from hatching until 5 weeks post-hatch. The continuous flow system (CFS) was used to create a dispersion of Goliat crude oil in seawater (Sanni *et al.*, 1998). The 5 mg/L oil dispersion was pumped into two mixing flasks and diluted to 0.010 mg/L and 0.015 mg/L (nominal oil concentrations). Each mixing flask had an overflow and 7 outlets, one for each replicate aquarium. Figure 3 show a drawing of the experimental set-up.

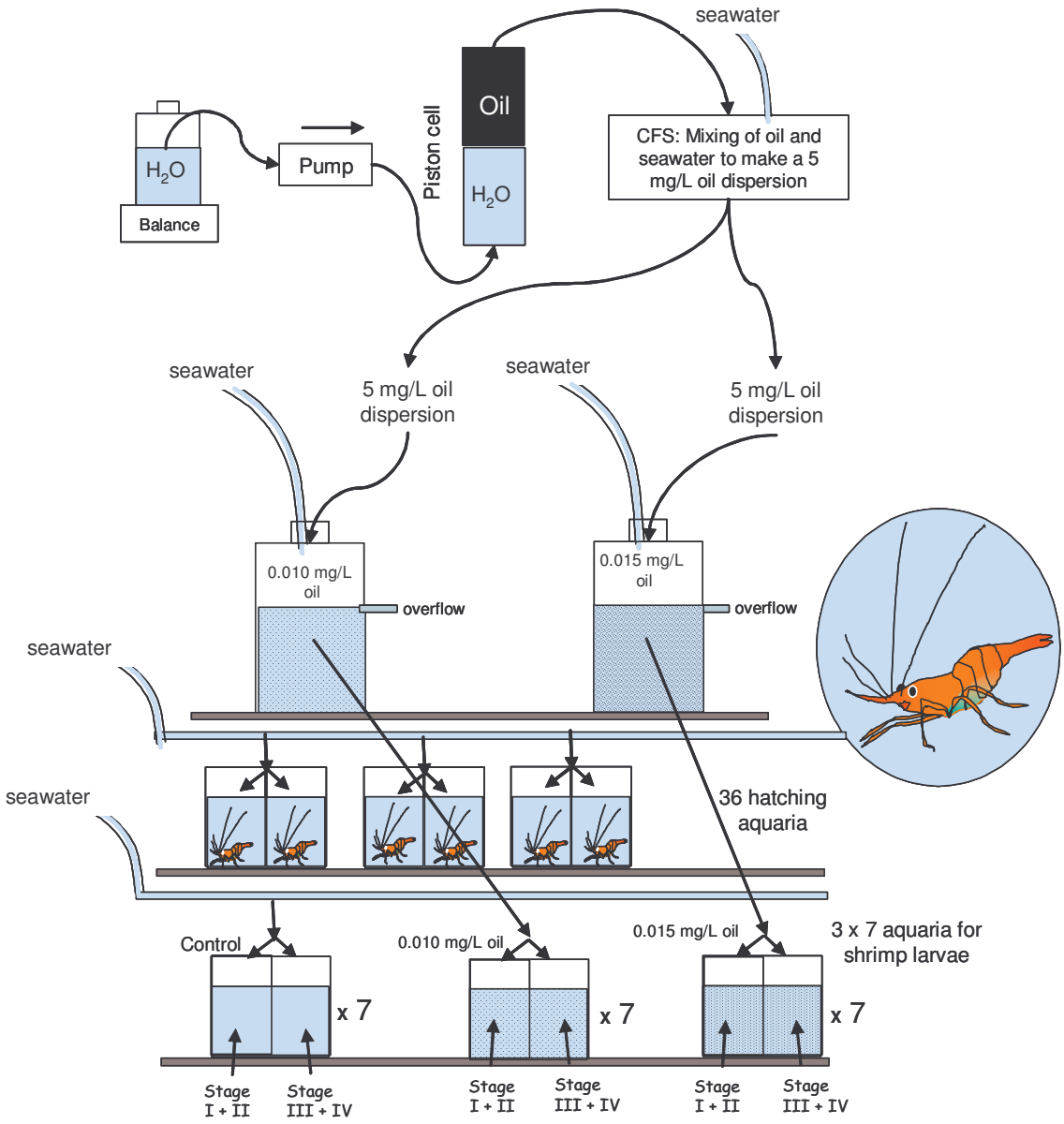

Figure 3. Experimental set-up for shrimp larvae exposed to Goliat oil. Adult females were kept in a separate rack.

Figure 4.

The oil dispersion was made by injection of oil into a flow of seawater using a precision pump (Figure 4). The pump delivered a constant flow of water into the lower part of a piston cell and oil was pushed out on the other side. To control that the flow of oil was constant throughout the experiment, the bottle of water was placed on a balance and the weight of the bottle was recorded daily. The results from the recording of weight showed that the input of oil was as planned: the mean deviation from the nominal flow of oil was only 2 %.

The 5 mg/L oil dispersion coming from the CFS was diluted with seawater in two mixing flasks (Figure 5) to obtain the two desired nominal concentrations (0.010 and 0.015 mg/L oil). The flow of seawater and dispersed Goliat oil into the two mixing flasks was measured every weekday (Appendix 1).

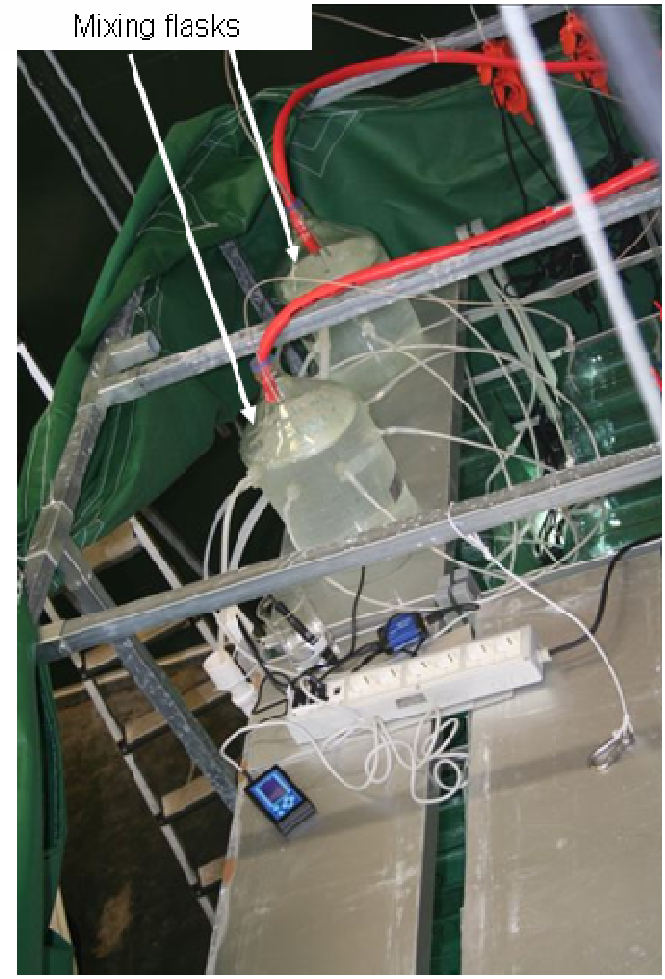

Figure 5. The mixing flasks where seawater and 5 mg/L oil dispersion is mixed to make 0.010 mg/L and 0.015 mg/L oil.

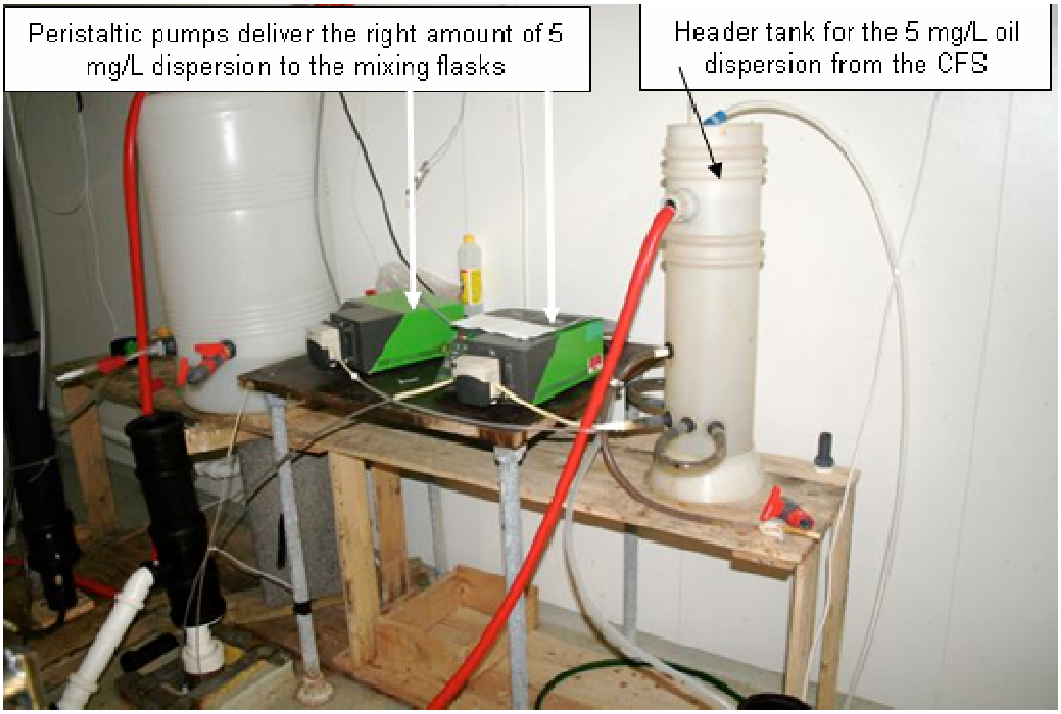

Figure 6. Peristaltic pumps and headertank for the 5 mg/L oil dispersion.

Two Watson Marlow peristaltic pumps (Figure 6) were used for pumping 5 mg/L oil dispersion from the CFS into each mixing flask. For the 0.010 mg/L solution the WM505S pump was set to 27 rpm, giving a mean measured flow rate of 8.2 ml/min (st. dev. ± 0.1, nominal flow: 8.4 ml/min). The mean measured flow of seawater into the 0.010 mg/L flask was 3.8 L/min (st. dev. ±0.2, nominal flow: 3.8 L/min) (Appendix 1).

For the 0.015 mg/L solution the WM505U pump was set to 6 rpm giving a mean measured flow rate of 13.7 ml/min (st. dev. ±0.2, nominal flow: 13.5 ml/min). The mean flow of seawater into the 0.015 mg/L flask was 4.0 L/min (st. dev.: ±0.2, nominal flow = 4.1 L/min) (Appendix 1).

Figure 7. Overview of the set up for the hatching aquaria.

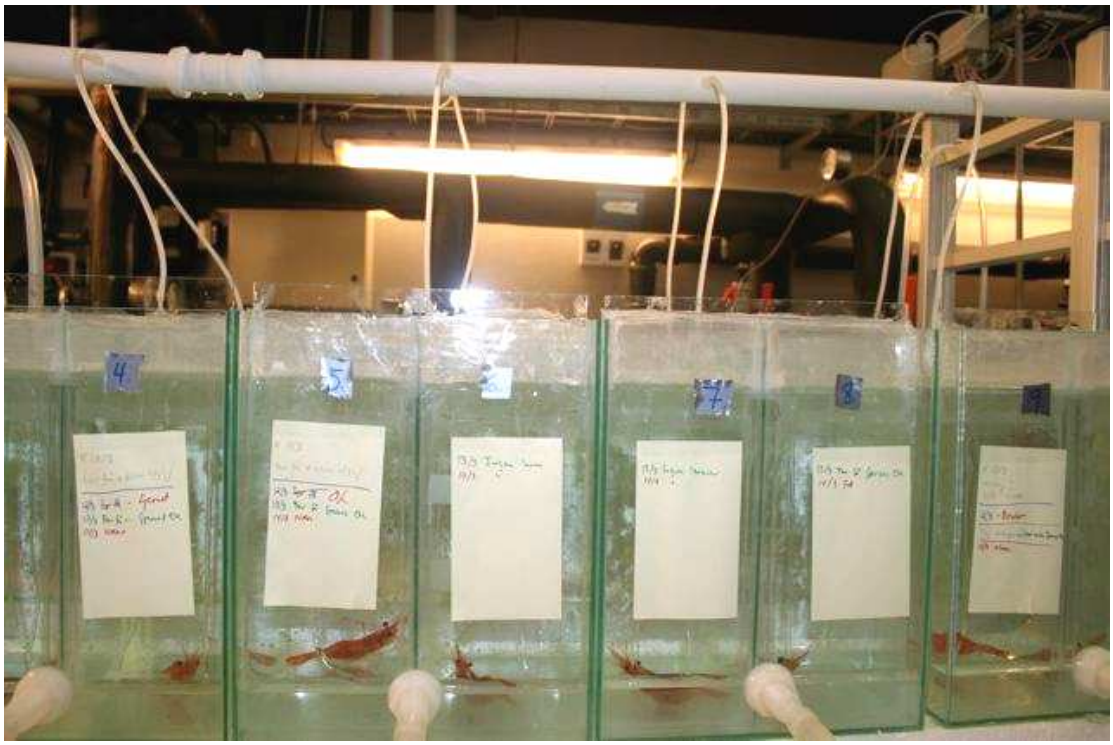

Figure 8. Two compartment hatching aquaria with one female in each compartment.

Female shrimps with embryos that were close to hatching were transferred from the quarantine tanks to glass aquaria (25 cm x 25 cm x 30 cm, volume 19 L, Figure 7 and 8). Each aquarium had two compartments, and one female shrimp was placed in each compartment. A total of 36 hatching aquaria were used. The aquaria outlets were equipped with plankton net covering to prevent loss of larvae.

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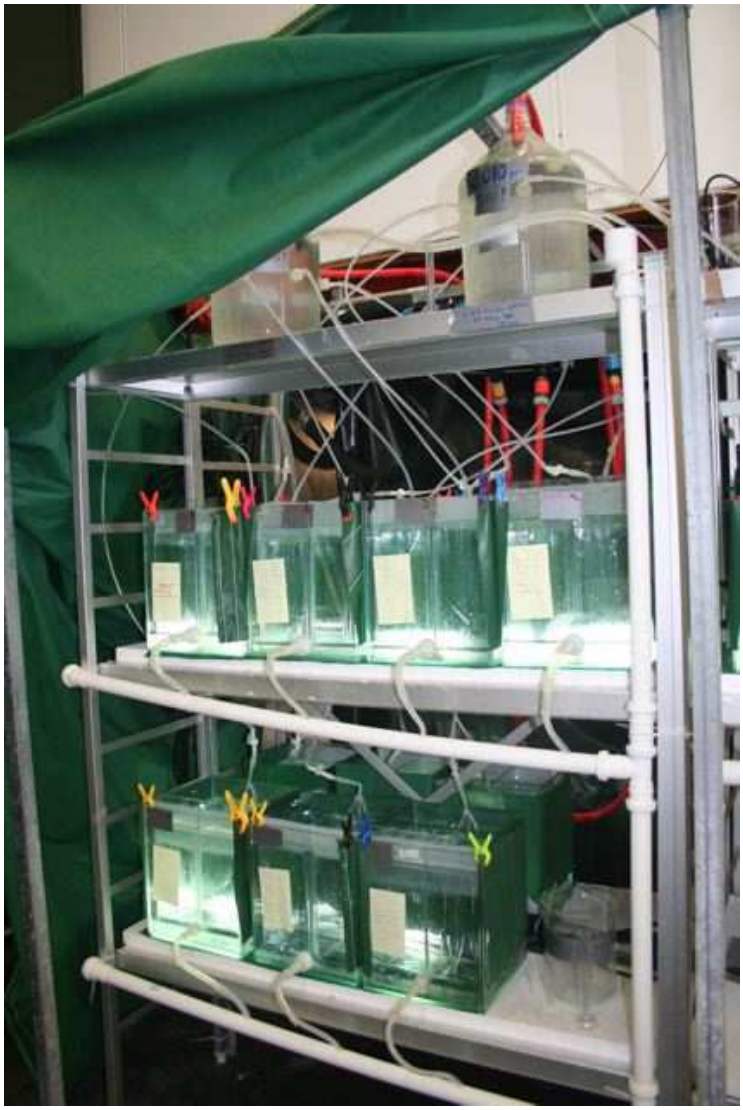

Figure 9. Experimental set-up with the two compartment aquaria

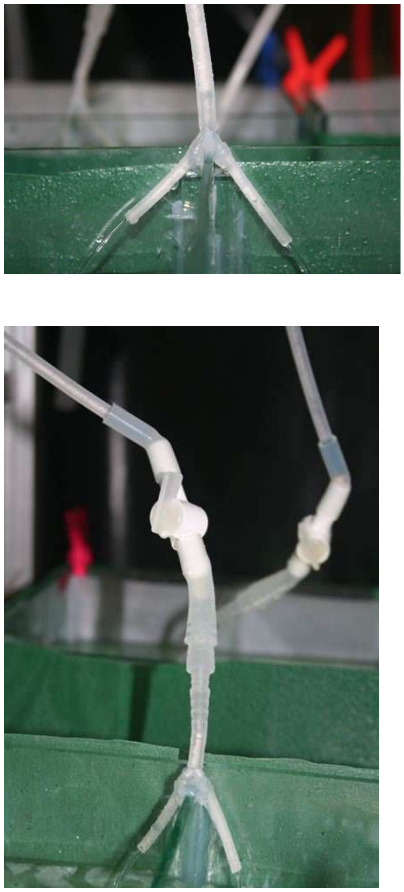

Figure 10. The split inlet of water/water and dispersed oil to each aquarium.

Figure 10 show the split inlet of water/water and dispersed oil to each aquarium. The rest of the system was similar to the 2007 shrimp larvae experiment (Figure 9).

After hatching, the larvae were kept in the same type of aquaria as the mothers. 21 aquaria were used for the larvae experiments. Each larvae aquarium had two equal size compartments (25 cm x 25 cm x 30 cm, volume: 19 L) with a flow-through water supply (Figure 9 and 10). The mean measured flow rate into each compartment of the aquaria was 122 ml per minute (st. dev. ±18, Appendix 2). Approximately 300 shrimp larvae were counted and transferred to each of the 21 aquaria (left compartment); 7 replicate aquaria for each test concentration (control, 0.010 mg/L oil and 0.015 mg/L oil). Details on the exact number of larvae in each aquarium are given in Appendices 5-

7. The larvae in each aquarium were a mixture of the offspring (< 24 hour old) from 3 mothers.

All larvae aquaria were surrounded by a non-transparent plastic drape to provide low light conditions (as in the 2007 BioSea II shrimp larvae experiment). Shrimp larvae show strong positive photo taxi, and will immediately swim towards any light source. This means that with normal daylight, or a light source in the ceiling, they will swim towards the surface and risk being trapped in potential oil film. A light source was therefore positioned near the bottom of the aquaria to keep the larvae away from the surface. The luminous intensity was measured with a lux-meter in the air above each aquarium. The mean was 24 lux (st. dev. ± 10, Appendix 4).

Figure 11 (left). Temperature sensor in the right compartment of the control 1 aquarium.

Figure 12 (right). Display of temperature sensor.

During the experiment weekly measurements of temperature and oxygen in each aquarium were done, in addition to the daily registrations of temperature in one aquarium (Figure 11 and 12).

3.3 The exposure

PAH in the oil

Eni provided a batch of Goliat oil from "Kobbe" for the 2008 experiment. The oil was received week 6 (2008). The oil was analyzed (for PAH) 5. February 2008. The concentration of PAH in the oil was analysed using GC/MS, and the results compared to the Goliat oil used in BioSea I and in the 2007 experiment with shrimp larvae. Fingerprint analysis of the oil was also done to show the composition of the oil (n-alkanes, nC17/Pristane and nC18/Phytane ratio), and to compare with the Goliat oil used in the 2007 experiment.

Pre-test

Before shrimp larvae were added to the aquaria, water samples were taken and analyzed for PAH to check if:

1) The concentrations of PAH in the aquaria were above the detection limit for the method used (SPE/GC-MS).

2) The measured PAH concentrations were similar to the predicted PAH concentrations.

Two pooled samples were taken from the aquaria of each test concentration.

Size of oil droplets

The particle size distribution of oil droplets in the oil dispersion was measured three times during the experiment using a Coulter counter in samples of the 5 mg/L oil dispersion from the CFS. The Coulter counter measures particles in the size range 1.6 - 50 µm.

PAH in the water

Water samples for PAH analysis were taken from the larvae aquaria 17 March, 25 March, 1 April, 8 April and 15 April 2008. The samples (~5 L water) were extracted using the solid phase extraction (SPE) method. Because larger volumes can be extracted with considerably less effort using SPE procedure than using liquid/liquid extraction the SPE was used. The sampling and extraction procedure is described in A. Nævdal: Solid Phase Extraction of PAH in water SOP: IRIS/2.2-607.

To make a 5 L water-sample from each treatment ~0.7 L was sampled from each aquarium within that treatment (pooled sample). The concentrations of naphthalene and C2 naphthalene in samples from the control and the two oil exposures were compared with the non-parametric Wilcoxon test[4].

At one sampling time (1 April) additional samples for liquid-liquid extraction was taken and PAH was analysed for comparison.

3.4 Mortality

Dead shrimp larvae were removed from each larvae aquarium/compartment daily. The mean accumulated mortality was calculated for each day and compared statistically using the Wilcoxon test[3].

3.5 Development time

Based on the experience from the 2007 experiment we started to check for presence of stage III larvae on day 20 of the experiment. All the larvae (of each replicate) were siphoned out, and stage III larvae were transferred to the right hand side compartment while the remaining stage II larvae returned to the left compartment of the aquarium. The left compartment of each aquarium was subsequently checked every day for new stage III larvae, and these were counted and transferred to the right hand side compartment. This was done every day until all the larvae had developed to stage III (or died) or the experiment ended.

The number of days it takes to reach a 50/50 ratio of stage II/stage III was calculated. The results indicate whether the larval development is affected by the treatment.

Finally, at the end of the exposure the relative proportions of larvae at stage III and IV were determined. Differences in the percentage of larvae reaching stage IV within 35 days may indicate effects on their development.

The first four larval stages are quite easily distinguishable (Figure 13, Haynes 1979). The difference between stage I and II is obvious, due to the significant change in the appearance of the eyes, which become stalked in stage II. It is slightly more difficult to differentiate between stage III and stage II, but it can be done by looking at the tail. The tip of the tail consist of three parts in stage III, whereas the middle part is in one whole, almost heart shaped, piece in stage II (Figure 13). Stage III and IV larvae are also differentiated by the appearance of the tail, with a much larger endopodite of the uropode in stage IV than in stage III larvae. In addition, the larvae grow each time they change stage.

[3] The non-parametric Wilcoxon test (Wilcoxon One-way test, Chi square approximation, JMP 5-1). Nonparametric tests are useful to test whether group means or medians are located at the same level across groups. However, the usual analysis of variance assumption of normality is not made. Nonparametric tests use functions of the response variable ranks, called rank scores (Hajek 1969). *Wilcoxon rank scores* are the simple ranks of the data. The Wilcoxon test is the most powerful rank test for errors with logistic distributions. No assumption is made regarding the *shape* of the population distribution.

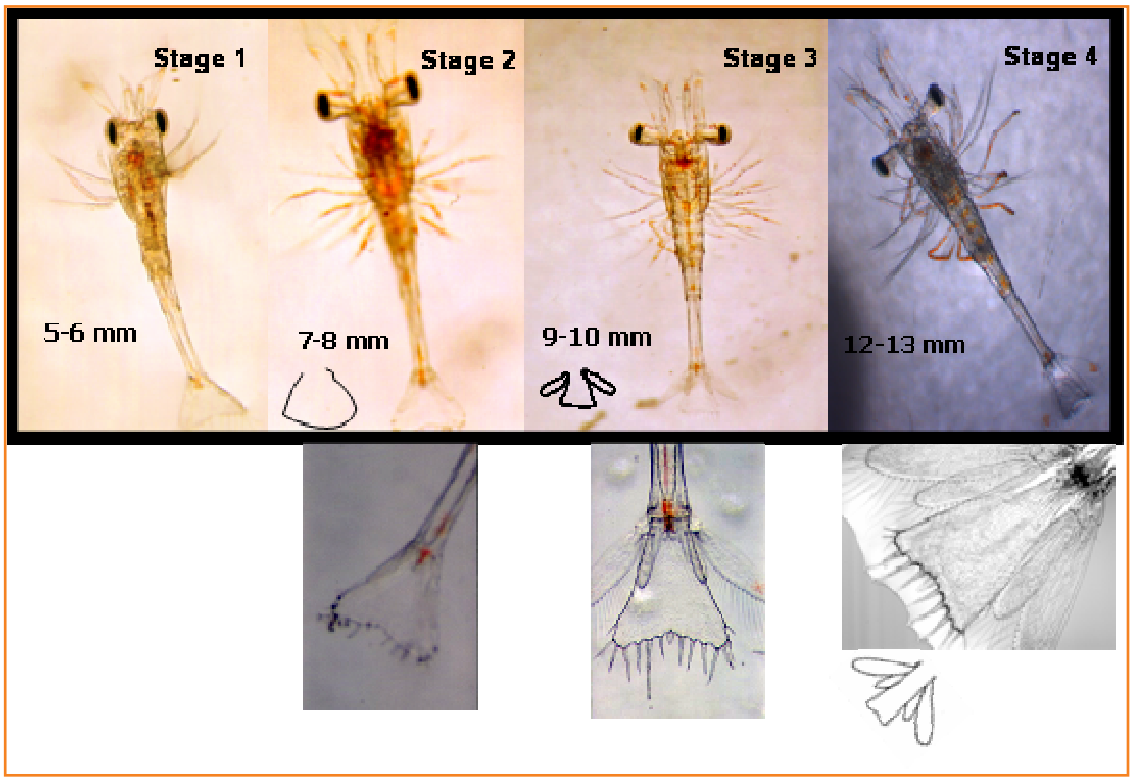

Figure 13. The four larval stages. Notice the change in appearance of eyes between stage I and II, and of the tail between stage II and III, - and between III and IV. The general shape of the tail is indicated by drawings and shown enlarged, below the larvae pictures. Based on information from Haynes 1979.

Figure 14. Shrimp larvae from the 2008 batch (collected from the quarantine tank) (Photo: A. H. Tandberg).

3.6 Growth

At the end of the exposure all surviving stage IV larvae were counted and pooled into four samples, and the wet weight was recorded. Then the larvae were dried in a drying cabinet at 60ºC for 24 hours and weighed again to obtain the dry weight. The mean wet and dry weight per shrimp larvae was calculated and the mean wet weight from the different treatments at the end of the experiment was compared using the non-parametric Wilcoxon test.

4 Results

4.1 Test conditions

Oxygen. The mean percentage of oxygen in the test chambers was 91 (st. dev. ± 4), and the mean concentration of oxygen in the test chambers was 11.4 mg/L (st. dev. ± 0.5) (Appendix 3).

Temperature. Temperature was measured with the oxygen-meter at the same time as the oxygen. The mean temperature in the test chambers was 5.0°C (st. dev. ± 0.2). The mean temperature that was logged in one compartment of the control 1 aquarium was also 5.0°C (st. dev. ± 0.2) (Appendix 3).

Salinity. The mean salinity in inlet water was 34.6 parts per thousand (st. dev. 0.9).

Oil dispersion. The size distribution of oil droplets was measured by Coulter counter in samples of the 5 mg/L oil dispersion coming from the CFS. Mean droplet size based on the volume of particles in each size category was 12 µm (st. dev. ± 1) (n = 21 measurements, samples taken 17 and 31 March and 8 April 2008, volume of each sample 0.5 ml) (Figure 15).

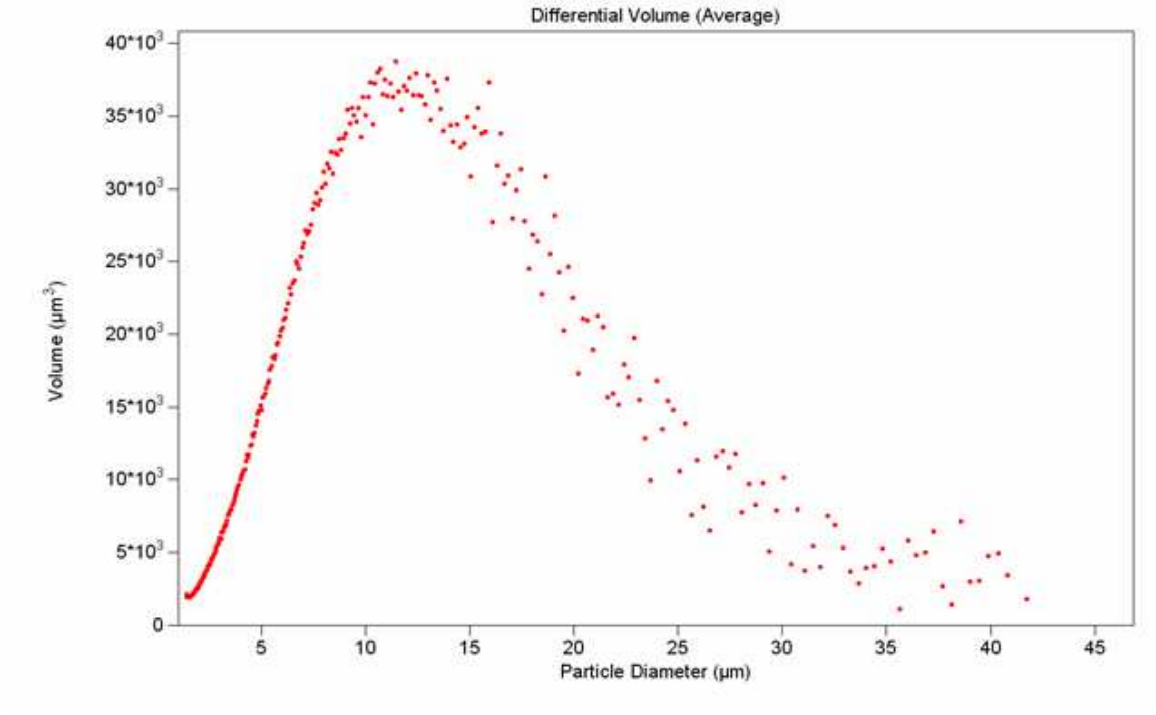

Figure 15. The size distribution of oil droplets measured by Coulter counter in samples of the 5 mg/L oil dispersion from the CFS.

4.2 Chemical analysis of PAH

PAH concentration of Goliat oil

Figure 16 shows the concentration of PAHs in the batch of Goliat oil provided by Eni in 2008, and Figure 17 shows the chromatograms for the Goliat oil used in the 2007 and 2008 shrimp larvae experiments. The 2008 oil contained more of the lighter oil fractions than the oil used in 2007, e.g. n-alkanes C9-C14. The nC17/Pristane ratio was different in the two batches of oil.

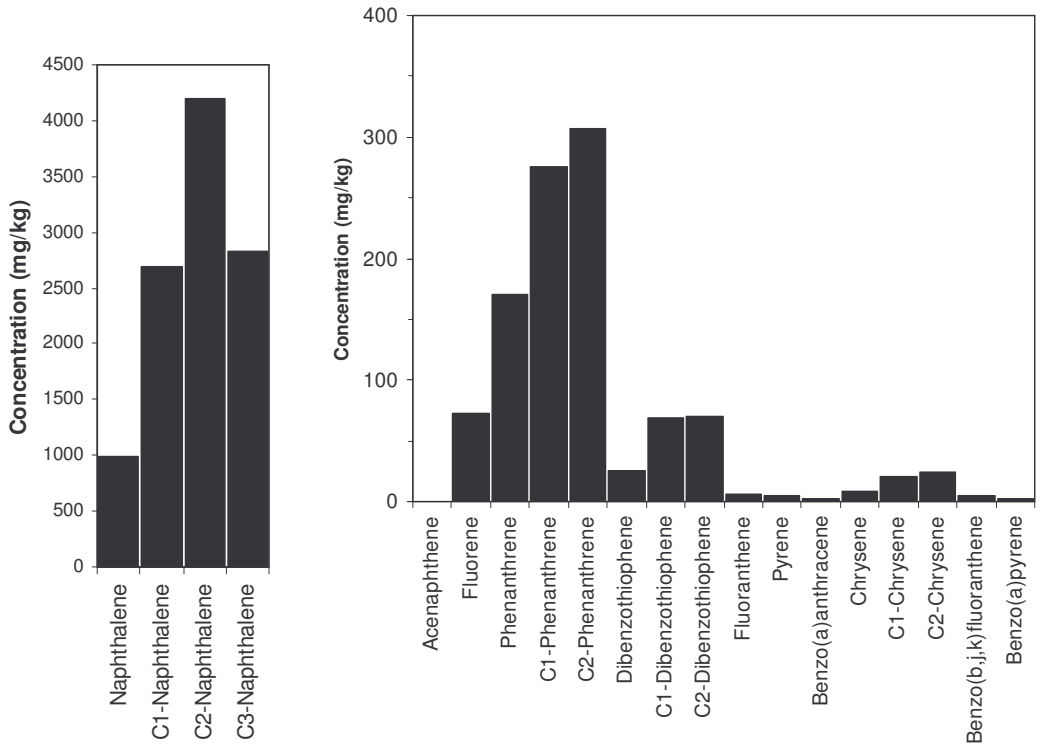

Figure 16. The mean concentration of C0-C3 naphthalenes (*left)* and 3 – 5 ring PAHs *(right*) in the batch of Goliat oil used in the 2008 experiment (analysed 5 February 2008). No 6-ring PAH was recorded above the quantification limit in the Goliat oil.

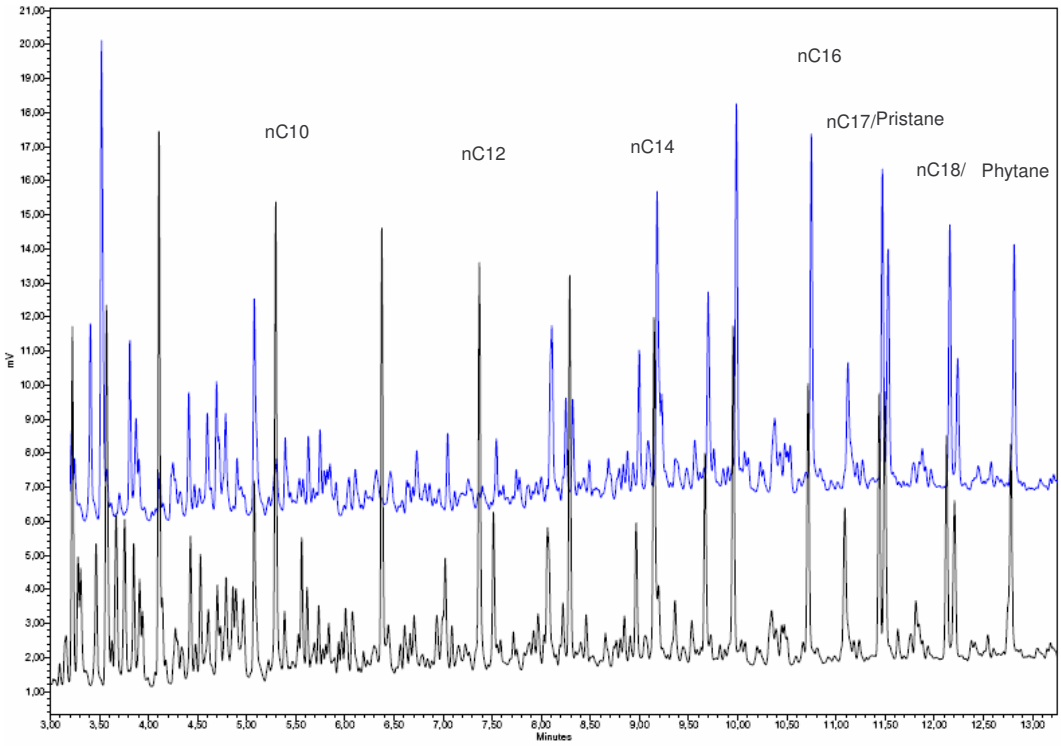

Figure 17. The figure show chromatogram of the Goliat oil used in the 2007 experiment (blue line) and the oil used in the 2008 experiment (black line). The figure shows the difference in the C9-C19 range only.

THC in oil dispersion from CFS

The nominal concentration of oil from the CFS was 5 mg/L (7 L/min seawater and 0.042 ml/min oil). One sample of 5 mg/L solution was analysed every week of the experiment. The mean measured concentration of THC in samples from the header-tank was 4.8 mg/L (st. dev: 0.4). The flows of this solution and seawater into the two mixing flasks (Figure 3 and 5) were measured (Appendix 1) and calculations of actual concentrations based on these measurements (THC in oil dispersion and flow rates) indicate that the actual oil concentrations in the aquaria were 0.0105 mg/L (5% higher than nominal) and 0.0163 mg/L (9% higher than nominal) (Table 2).

Table 2. Estimation of actual oil concentrations based on THC in the oil dispersion and flows into the mixing flasks.

Nominal concentration: 0.010 mg/L oil		**Nominal concentration: 0.015 mg/L oil**	
4.8	mg/L oil in dispersion from CFS	4.8	mg/L oil in dispersion from CFS
8.2	ml/min oil dispersion	13.7	ml/min oil dispersion
0.04	mg oil in 8.2 ml/min oil dispersion	0.07	mg oil in 13.7 ml/min oil dispersion
3.76	L/min seawater	4.04	L/min seawater
0.0105	mg oil pr liter seawater	0.0163	mg oil pr liter seawater
105	i.e. 5 % higher than nominal oil concentration	109	i.e. 9 % higher than nominal oil concentration

PAH concentration in water from the exposure tanks

Results from the pre-test

The measured concentrations of PAHs in the two replicate samples from the exposure aquaria (A and B, Table 3) from each concentration were almost identical. The measured concentrations of PAHs were very similar to the predicted PAH concentrations (Table 3).

The predicted PAH concentration (Table 3) in 0.015 mg/L oil in the 2008 experiment was based on the measured concentrations of PAH in the 0.015 mg/L exposure aquaria in 2007 and the difference in PAH concentration between the 2007 and 2008 Goliat oil:

$$\text{Predicted concentration of PAH in the 0.015 mg/L aquaria in 2008} = \text{PAH in 0.015 mg/L aquaria in 2007} \; / \; \left(\frac{\text{PAH in 2007 oil}}{\text{PAH in 2008 oil}} \right)$$

The predicted PAH concentration (Table 3) in 0.010 mg/L oil in the 2008 experiment should be 33% lower than the values predicted for the 0.015 mg/L oil treatment. Details of the calculations are given in Appendix 12.

Table 3. Predicted and measured concentrations (µg/L) of PAH in the water in the pre-test. The samples were taken before shrimp larvae were added to the aquaria.

		Measured PAH in pre-test			Measured PAH in pre-test	
µg/L PAH	Predicted 0.010 mg/L	A 0.010 mg/L	B 0.010 mg/L	Predicted 0.015 mg/L	A 0.015 mg/L	B 0.015 mg/L
Naphthalene	0.011	0.012	0.011	0.016	0.017	0.017
C1-Naphthalene	0.019	0.023	0.024	0.029	0.038	0.038
C2-Naphthalene	0.040	0.037	0.037	0.060	0.055	0.053
C3-Naphthalene	0.028	0.021	0.021	0.042	0.033	0.031
Fluorene		0.001	0.001		0.001	0.001
Phenanthrene	0.001	0.002	0.002	0.002	0.003	0.003
C1-phenanthrene		0.002	0.002		0.003	0.003

Results from the experiment

The mean concentrations of PAHs in the exposure tanks are shown in Figure 18 and Table 4, and the composition (%) of the different PAHs are given in Table 5.

- The mean measured concentration of sumPAH in the 0.010 mg/L and 0.015 mg/L exposure was 32 % and 19 % lower, respectively, than in the pre-test (Table 3 and 4).
- There was a significant difference in the mean concentration of PAHs between control, 0.010 mg/L and 0.015 mg/L Goliat oil (Wilcoxon, $p<0.05$). Figures 18, 19 and 20 illustrate the difference.
- Significantly lower concentration of PAHs was found in the 0.010 mg/L treatment than in the 0.015 mg/L treatment. The mean measured concentration of PAHs in 0.010 mg/L oil was 47 % lower than in the 0.015 mg/L oil exposure.
- The concentration of PAHs in the 0.010 mg/L treatment was significantly higher than the background/control level.
- The exposure concentrations were constant with time (Appendix 11).
- Approximately 90 % of the PAHs in the exposure tanks were C0-C3 naphthalenes and the rest were 3-ring PAHs (Table 5).

We tried to estimate the actual oil concentrations using two different approaches: 1) the THC concentration in the dispersion and flow rates into the mixing flasks (Table 2), and 2) using PAH in the oil and in the water (Table 6).

In Table 6 the nominal concentrations of PAHs are compared to the measured concentrations. The box below shows the formula for calculating the nominal

concentration of PAH in the exposure aquaria based on the measured concentration of PAH in the oil. See footnote about dilution factors for oil[4], and details in Appendix 13.

$$\text{NOMINAL concentration (µg/L) of PAH in the aquaria in 2008} = \frac{\text{µg PAH in the 2008 oil}}{\text{Dilution factor for the oil}^4}$$

- The estimations of oil concentrations based on flows of oil dispersion and seawater in to the system and the concentration of THC in the dispersion indicated that the actual oil concentration was 5-9% higher than the nominal (Table 2). The estimation based on PAH concentration in the oil and in the water of the exposure tanks, however, indicate that the actual concentrations of oil were lower than the nominal (Table 6).

- The measured concentrations of PAHs were in general lower than the nominal concentrations (Table 6).

- There was a larger difference between nominal and measured concentrations for C0-C2 naphthalene than for the other PAH compound (Table 6).

Atle Nævdal (IRIS-Biomiljø, *pers. comm.*) recommended using the concentration of phenanthrene when comparing nominal to measured concentrations. The other PAHs that were detectable both in the oil and in the aquaria can also be used to get an indication of the actual oil concentrations in the experiments.

- Based on the phenanthrene level in the oil and in the aquaria the actual oil concentration in the 0.015 mg/L treatment was ca 20 % lower than the nominal (that is: 0.012 mg/L), and the actual oil concentration in the 0.010 mg/L treatment was 30 % lower than the nominal (0.007 mg/L) (Table 6).

Any calculation of actual oil concentration in the aquaria will only be indicative. The two ways of calculating actual oil concentrations above indicate, however, that the oil concentrations were close to the nominal level.

The most important information is the measured concentrations of PAH in the aquaria in the different treatments and experiments. These concentrations and the observed effects can be compared between experiments (e.g. BioSea I and II).

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[4] There is an error in the oil dilution factors given on page 22 in the shrimp larvae report from 2007 (Taban *et al*., 2007). Below are the nominal oil dilution factors for the 2007 and the 2008 experiment. ”Pure" oil = 1000 000 mg oil (1 kg oil per kg water). In the experiments the following concentrations were tested: 0.010 mg, 0.015 mg and 0.06 mg oil per kg water.

Nominal dilution factors for oil:

- 0.010 mg/L: 1000 000/0.010 = 100 000 000 = 100 million times diluted oil
- 0.015 mg/L: 1000 000/0.015 = 66 666 667 = 67 million times diluted oil
- 0.060 mg/L: 1000 000/0.06 = 16 666 667 = 17 million times diluted oil

Table 4. Mean concentration (± st. dev) of PAHs (µg/L) in water samples from the exposure tanks. The samples were taken weekly (n = 5). The dibenzothiophenes and all 4 – 6 ring PAHs were below the quantification limit in the exposure tanks. The SPE extraction method was used for the PAHs.

µg/L	Control	0.010 mg/L Goliat - Kobbe oil	0.015 mg/L Goliat - Kobbe oil
Naphthalene	0.002 ± 0.0003	0.005 ± 0.0014	0.010 ± 0.0013
C1-Naphthalene	0.003 ± 0.0014	0.013 ± 0.0010	0.027 ± 0.0016
C2-Naphthalene	0.005 ± 0.0015	0.024 ± 0.003	0.043 ± 0.0033
C3-Naphthalene	0.005 ± 0.0005	0.020 ± 0.0026	0.034 ± 0.0024
sum 2 ring PAH	**0.017**	**0.062**	**0.114**
Fluorene		0.001	0.001 ± 0.0001
Phenanthrene		0.001 ± 0.0002	0.002 ± 0.0001
C1-Phen/Anthr		0.002 ± 0.0001	0.003 ± 0.0002
C2-Phen/Anthr			0.003 ± 0.0003
sum 3-ring PAH	**-**	**0.004**	**0.010**
Sum PAHs	**0.02**	**0.07**	**0.12**

Table 5. Relative composition of PAH groups in the Goliat-Kobbe oil and in water samples from the exposure tanks. Each group's contribution (percentage) to the measured sums of PAHs. Only the PAH compounds that were above the quantification limit are included.

	Percent of each group of PAH			
	Goliat-Kobbe oil	**0.010 mg/L**	**0.015 mg/L**	Compounds
2 ring PAHs	91.0	93.8	91.9	C0-C3 naphthalenes
3 ring PAHs	7.0	6.2	8.1	Acenapthene, fluorene, C0-C2 phenanthrene
DBTs	1.4	-	-	C0-C2 dibenzothiophenes
4 ring PAHs	0.6	-	-	Fluoranthene, Pyrene, Benzo(a)anthracene, C0-C2 chrysene
5 ring PAHs	0.1	-	-	Benzo(b,j,k)-fluoranthene, Benzo(a)pyrene

Table 6. The nominal concentration of PAH divided by the measured concentrations of PAH in the 2008 shrimp larvae experiment. If the value is = 1 the measured PAH concentration is the same as the nominal. If the value is 2 the measured concentration is 50% lower than the nominal. If the value is 0.5 the measured concentration is twice as high as the nominal concentration (See appendix 13 for details).

Nominal concentration of PAH / Measured concentrations of PAH		
	0.010 mg/L Goliat/Kobbe (2008) Nominal conc./ Measured conc.	0.015 mg/L Goliat/Kobbe (2008) Nominal conc./ Measured conc.
Naphthalene	2.0	1.4
C1-Naphthalene	2.1	1.5
C2-Naphthalene	1.7	1.5
C3-Naphthalene	1.4	1.3
Fluorene	0.8	0.9
Phenanthrene	1.3	1.2
C1-Phenanthrene	1.5	1.2

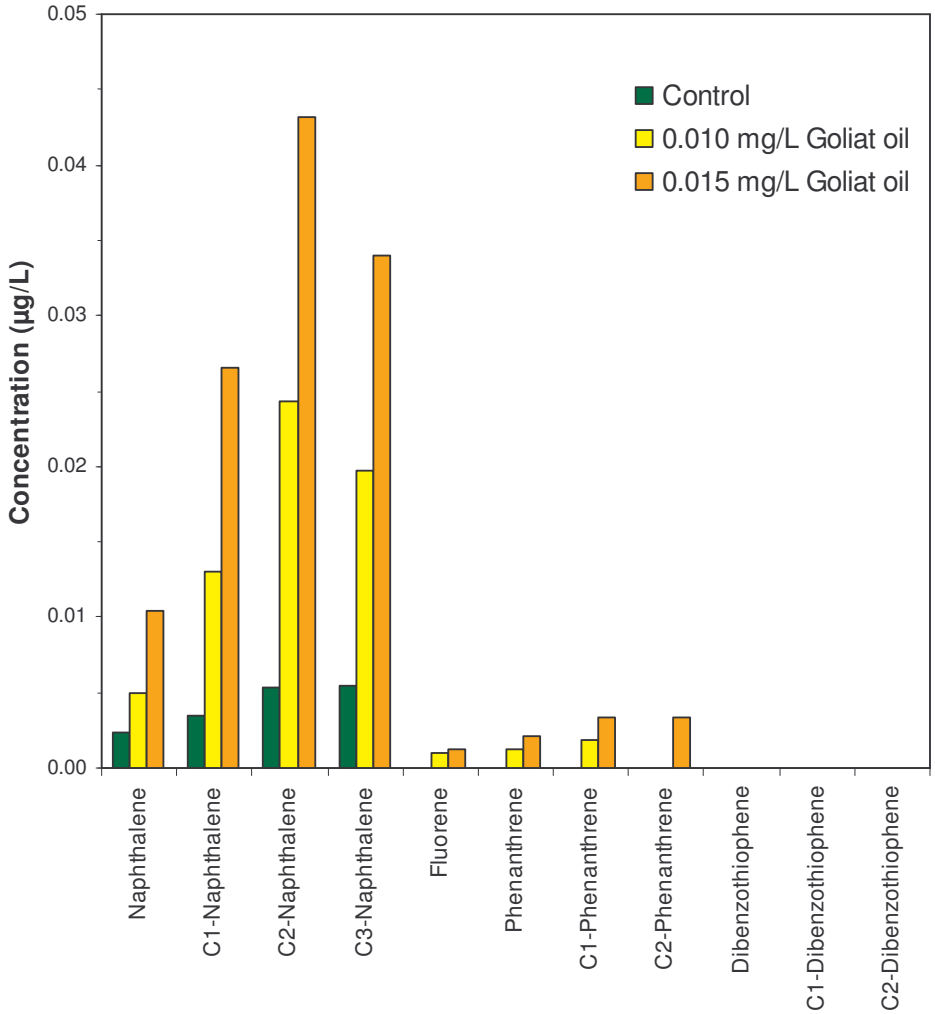

Figure 18. Concentration of PAH in the exposure tanks. Only C0-C3 naphthalenes, fluorene, C0- and C1 phenanthrene were detectable in both dilutions of Goliat oil.

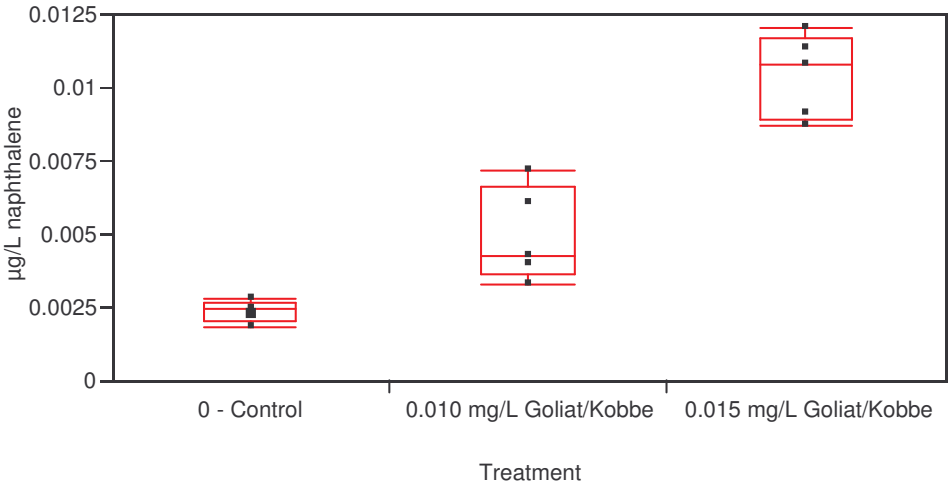

Figure 19. Comparison of measured concentrations of naphthalene in control and the two oil exposures (n = 5 replicate samples, one from each week of the experiment)[5].

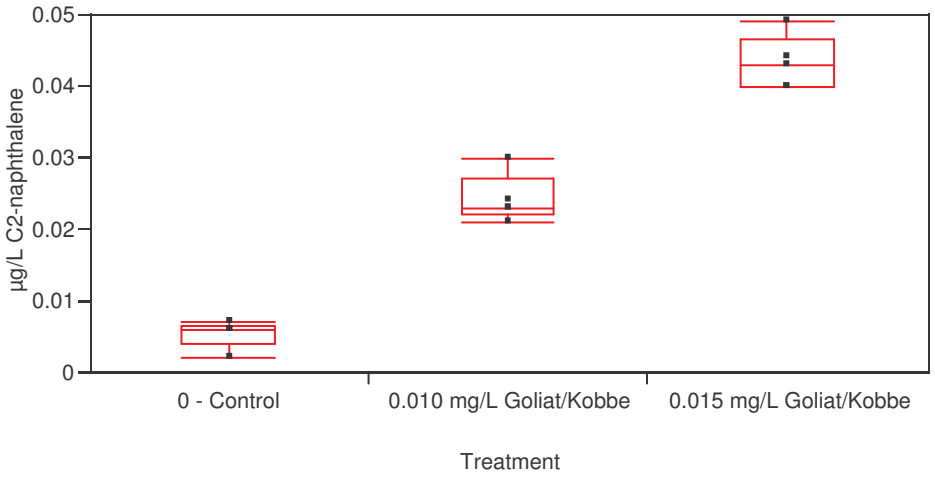

Figure 20. Comparison of measured concentrations of C2-naphthalene in control and the two oil exposures (n = 5 replicate samples, one from each week of the experiment). C2-naphthalene was the PAH compound that reach the highest concentrations in the water.

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[5] Box plots (from JMP 5-1): The ends of the box are the 25th and 75th quantiles. The difference between the quartiles is the *interquartile range*. The line across the middle of the box identifies the median sample value. Each box has lines, sometimes called *whiskers*, that extend from each end. The whiskers extend from the ends of the box to the outermost data point that falls within the distances computed:

upper quartile + 1.5*(interquartile range)
lower quartile - 1.5*(interquartile range).

Comparison of PAH analysis on samples extracted using SPE and liquid-liquid

Larger volumes of water can be extracted with considerably less effort using SPE procedure than using liquid/liquid extraction. Therefore, SPE was used for PAH analysis on the water samples collected each week of the experiment. Since the L/L extraction method was used in BioSea I and earlier experiments, we included one sampling time with both extraction methods and compared the results from the analysis of PAH in the extracted samples.

Only naphthalenes were above the quantification limit in samples extracted using L/L. The concentration of naphthalenes was almost the same in samples extracted with L/L and with SPE (Table 7). More of the larger PAHs were above the quantification limit using the SPE than L/L, because a larger volume is extracted. Fluorene and C0-C2 phenanthrene were above the quantification limit using SPE for water samples from the 0.015 mg/L exposure, but the concentrations of these compounds were below the quantification limit for the liquid/liquid extraction method.

Table 7. Results from comparison of two extraction procedures for PAH from seawater samples. In week 3 (1 April) of the experiment, water samples were taken and extracted both with SPE and liquid/liquid extraction procedures.

µg/L	0.010 mg/L (SPE)	0.010 mg/L (Liquid/liquid)	0.015 mg/L (SPE)	0.015 mg/L (Liquid/liquid)
Naphthalene	0.006	*< (0.004)	0.011	0.010
C1-Naphthalene	0.013	0.011	0.029	0.027
C2-Naphthalene	0.023	0.020	0.049	0.048
C3-Naphthalene	0.020	*< (0.016)	0.035	0.035

Measured test concentrations *vs* field concentrations. If there is need to compare the tested concentrations to field levels we recommend to use the measured concentrations of PAH in the exposure tanks and compare these to those measured or modelled for the field situation.

4.3 Mortality

The mean accumulated mortality in the control group was 8 % 35 days post-hatch (Figure 21). The mortality in the two exposed groups of shrimps was 2-3 % higher than in the control after 35 days exposure (Figure 21). There was, however, no statistical difference between control and exposed groups (Wilcoxon, $p>0.05$, $n=7$).

Larvae that had developed into stage III were transferred to a separate compartment of the aquaria each day from 20 days post-hatch. The mortality of these stage III larvae was lower than the mortality of the stage II larvae (Appendix 5-7). Based on the average mortality per day (Figure 22) the mortality appeared to be highest before the larvae started to develop from stage II to stage III.

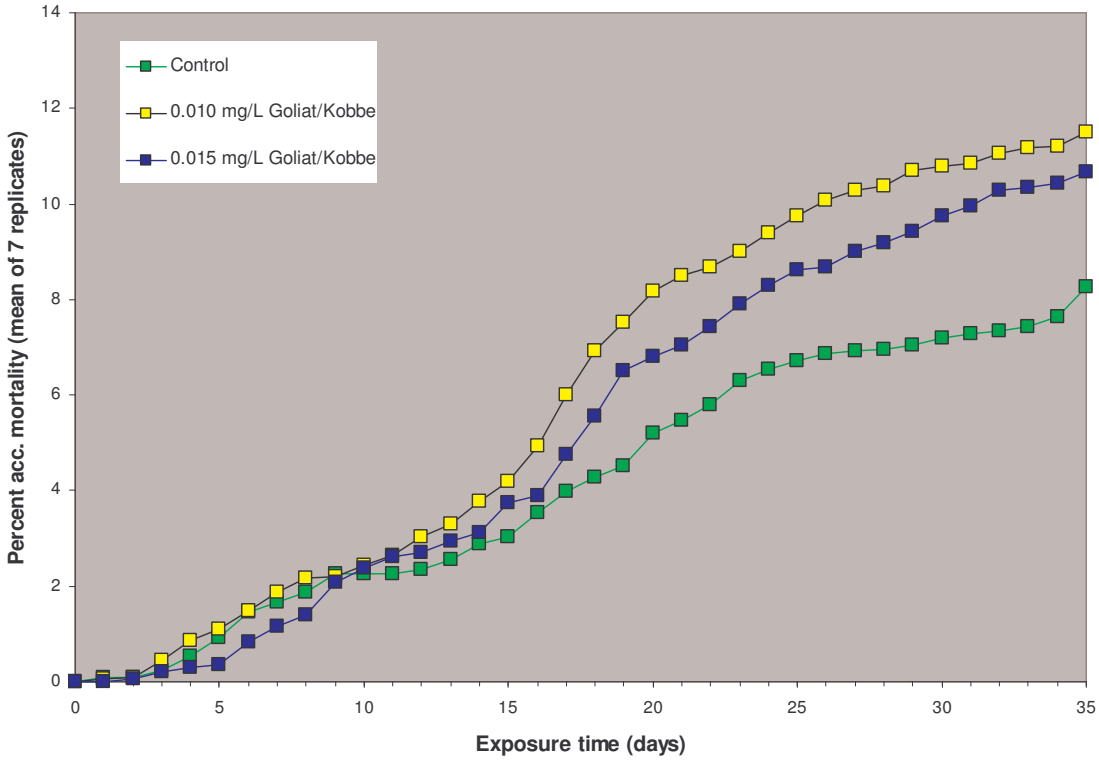


Figure 21. Mean accumulated mortality for shrimp larvae exposed to Goliat/Kobbe oil with increasing exposure time.

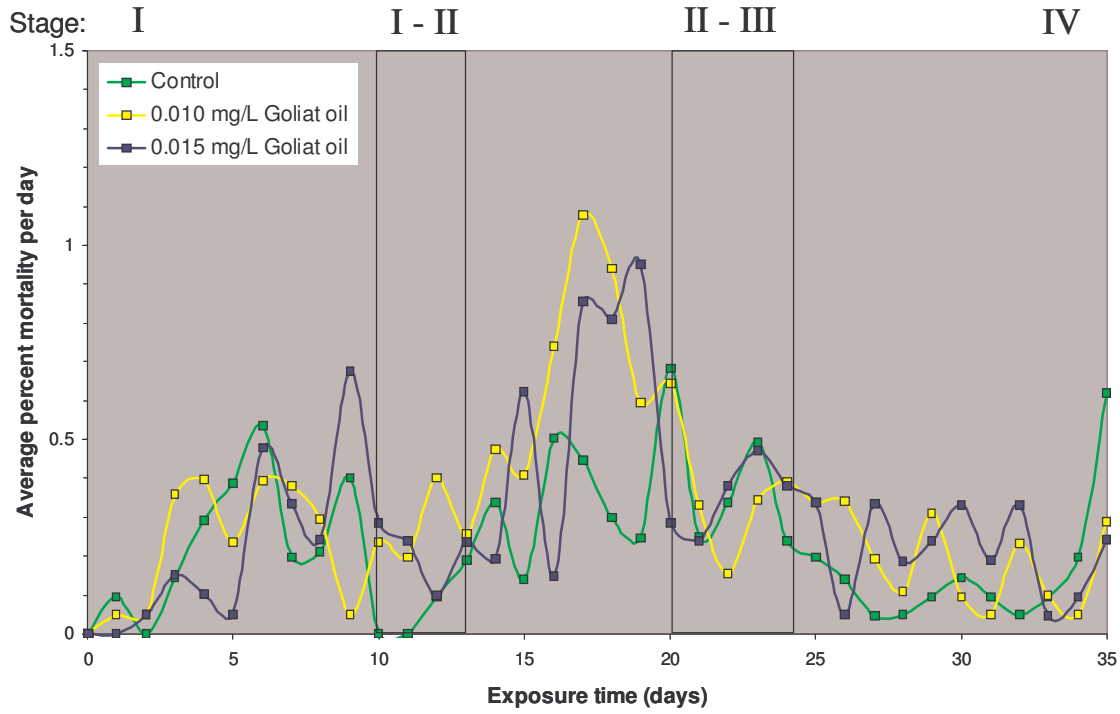


Figure 22. Average percent mortality of shrimp larvae per day (mean number of dead larvae for the 7 replicate aquaria n=7). The vertical lines show the period the larvae are developing from stage I to stage II and from stage II to stage III.

Conclusion - Mortality:

For shrimp larvae exposed to oil from hatching and until 35 days post-hatch the NOEC for mortality was 0.015 mg/L oil from Goliat/Kobbe (= the highest tested concentration).

4.4 Development time

From stage I to stage II. We observed the first empty exoskeletons at the bottom of the aquaria (0.010 mg/L replicate 2 and 0.015 mg/L replicate 1) after 10 days exposure (10 days post-hatch), indicating that the larvae had started moulting and developing into stage II larvae. During the next three days (day 11-13 post-hatch) most of the larvae had developed into stage II judging from the amount of exoskeletons rinsed from the bottom of each aquarium daily and from our observation of the larvae.

From stage II to stage III. The first larvae developed into stage III larvae 20 days post-hatch, and more than 95 % of the larvae had reached stage III on day 24 (Figure 23).

Median time to reach stage III was calculated and compared statistically using Wilcoxon test (Table 8). The comparison is based on the total number of larvae that reached stage III (not including those that died before they reached stage III). There was a statistically significant *decrease* in development time for larvae exposed to the lowest exposure concentration 0.010 mg/L (Table 8). There was no difference in development time (to stage III) for larvae exposed to 0.015 mg/L oil compared to the control.

Figure 24 shows the mean percent of stage III larvae at day 19 to 25 in the experiment based on the total number of larvae added to each aquarium at the start of the experiment. Figure 24 integrates mortality and development time. All larvae that survived long enough managed to develop to stage III.

In Table 8 the comparison of median time to reach stage III is based on the total number of larvae that reached stage III. Hence n is much higher (details in Table 8) and the small difference indicated between control and the low exposure in Figure 23 and 24 became statistically significant.

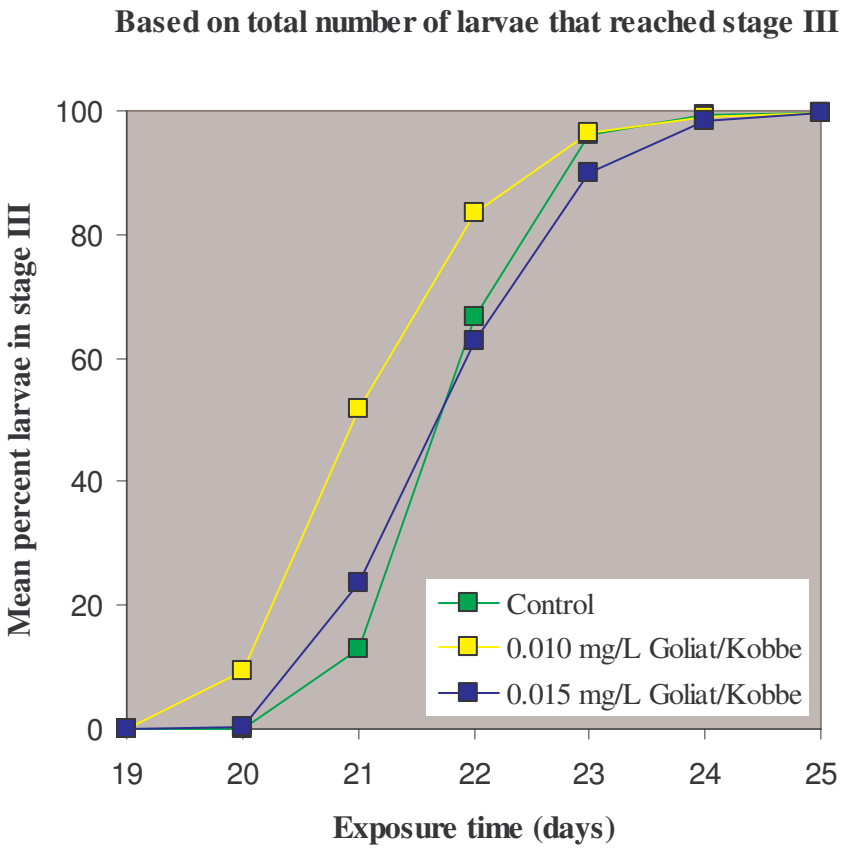


Figure 23. Mean percent stage III larvae of the total number of larvae that reached stage III in each treatment (n = 7).

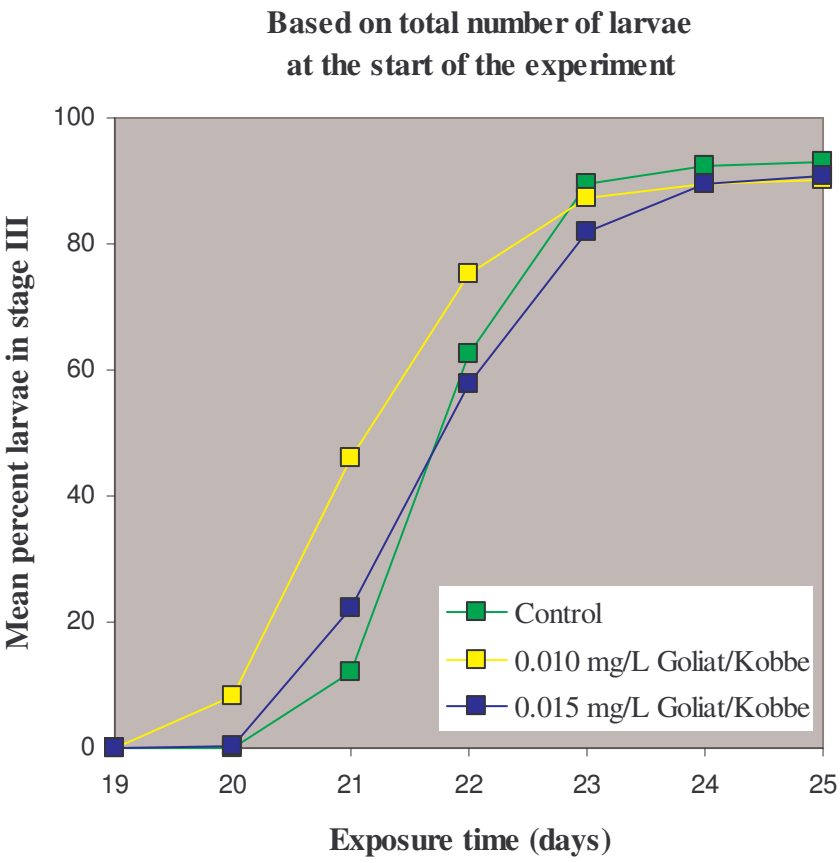


Figure 24. Mean percent stage III larvae day 19 to day 25 post-hatch in the experiment based on the total number of larvae added to each aquaria at the start of the experiment (n = 7).

Table 8. Development time for shrimp larvae exposed to Goliat oil. Median time to reach stage III (n = all larvae that reached stage III in each treatment: Control: n = 1872, 0.010 mg/L: n = 1848 and 0.015 mg/L: n = 1887).

Treatment	Days to stage III (median)	Wilcoxon
Control	22	-
0.010 mg/L Goliat oil	21	$p < 0.0001$
0.015 mg/L Goliat oil	22	$p = 0.6$

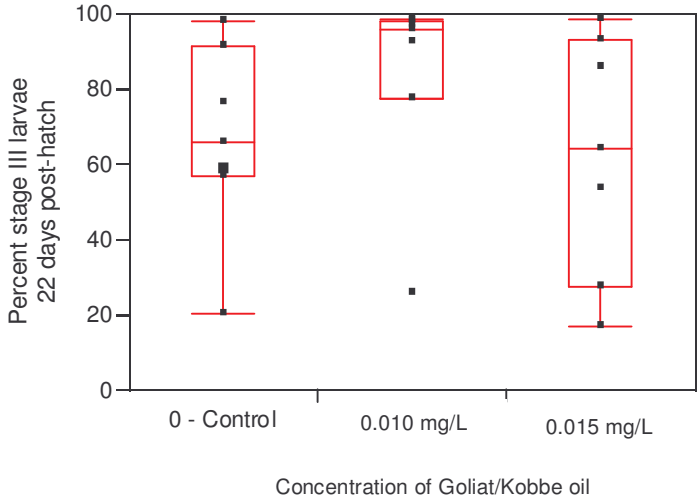


Figure 25. Percent stage III larvae 22 days post-hatch (mean percent stage III larvae 22 days post-hatch for the seven replicates from each treatment, n=7, was compared with Wilcoxon test). The median time to reach stage III for control larvae was 22 days.

From stage III to stage IV. Development time was also studied at the end of the experiment. The number of stage III and stage IV larvae was determined at the last day of the experiment (35 days post-hatch). Figure 26 shows that there was no significant difference in percent of larvae that had reached stage IV between the control larvae and the oil exposed larvae.

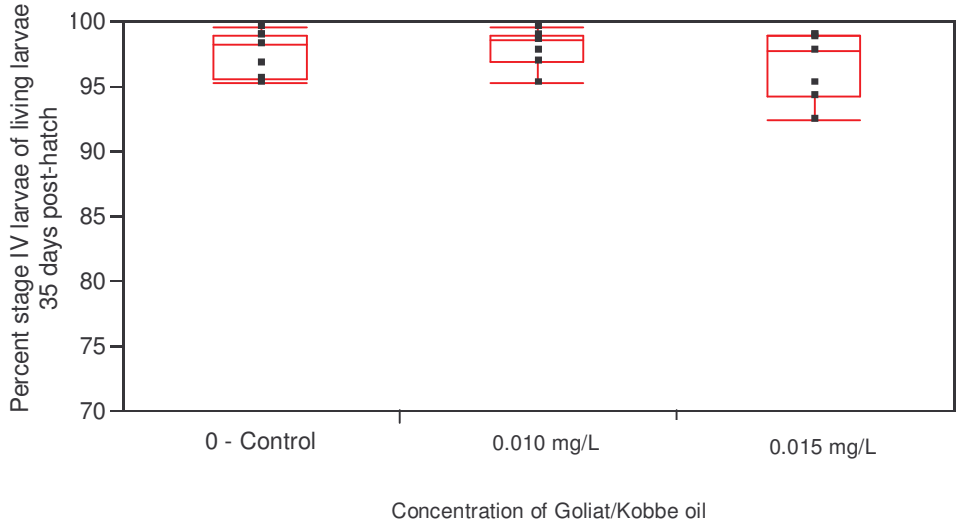


Figure 26. Percent stage IV larvae of the surviving larvae at the end of the experiment (mean percent larvae in stage IV for each of the 7 replicates, n=7, was compared with Wilcoxon test, $p > 0.05$).

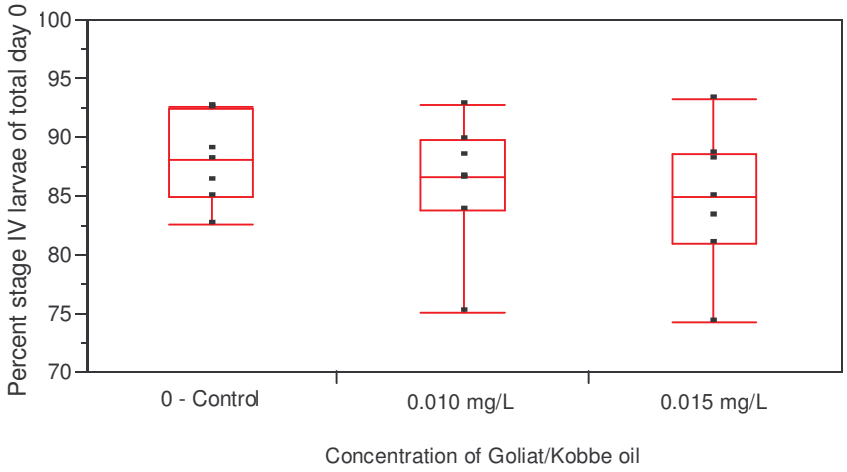


Figure 27. Percent stage IV larvae of the total number at start of the experiment (mean percent larvae in stage IV for each of the 7 replicates, n=7, was compared with Wilcoxon test, $p > 0.05$).

Stage II – stage III:

The development time from stage II to stage III was on average one day faster for larvae exposed to 0.010 mg/L Goliat/Kobbe oil than for control larvae. No effect on development time could be detected for larvae exposed to 0.015 mg/L Goliat/Kobbe oil.

Stage III – stage IV:

The percentage of larvae that developed to stage IV was not affected by 35 days exposure to 0.010 mg/L or 0.015 mg/L Goliat/Kobbe oil.

Conclusion – Development time:

Since no difference in development time was detected after 35 days exposure we conclude that the over-all NOEC for the Goliat/Kobbe oil related to development-time was 0.015 mg/L Goliat oil (= highest tested concentration).

4.5 Growth

There was no difference in mean wet weight or dry weight of shrimp larvae from the control and the oil exposed groups at the end of the experiment.

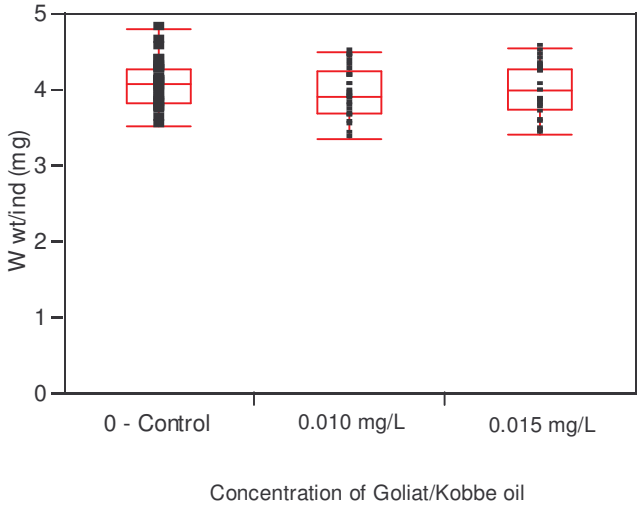


Figure 28. Mean individual wet weight (mg) of stage IV shrimp larvae at the end of the experiment 35 days post-hatch.

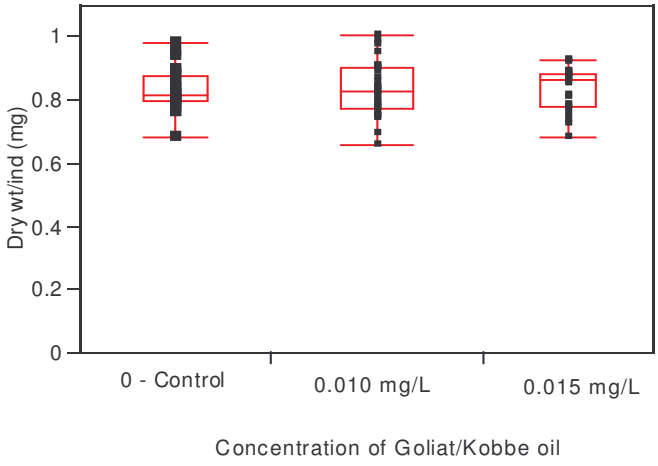


Figure 29. Mean individual dry weight (mg) of stage IV shrimp larvae at the end of the experiment 35 days post-hatch.

Conclusion - Growth:

The growth (weight) of shrimp larvae was not affected by 35 days exposure to 0.010 mg/L and 0.015 mg/L Goliat/Kobbe oil.

NOEC = 0.015 mg/L Goliat/Kobbe oil (= the highest tested concentration)

5 Discussion

The oil

We have compared the PAH concentration in the Goliat oil from Real (used in 2004 and 2007) and from Kobbe (used in 2008). The Goliat oil from Real was analysed twice. The two batches of oil were quite different (Figure 30). The Goliat/Real oil had:

- Approximately twice as much naphthalenes, fluorene, C1-phenanthrene and pyrene per kg oil as the Goliat/Kobbe oil (Figure 30 below).
- 4-5 times as much DBTs per gram oil as the Goliat/Kobbe oil.
- Less of the lighter oil fractions than the Goliat/Kobbe oil, e.g. n-alkanes C9-C14.
- The nC17/Pristane ratio was different in the two batches of oil.

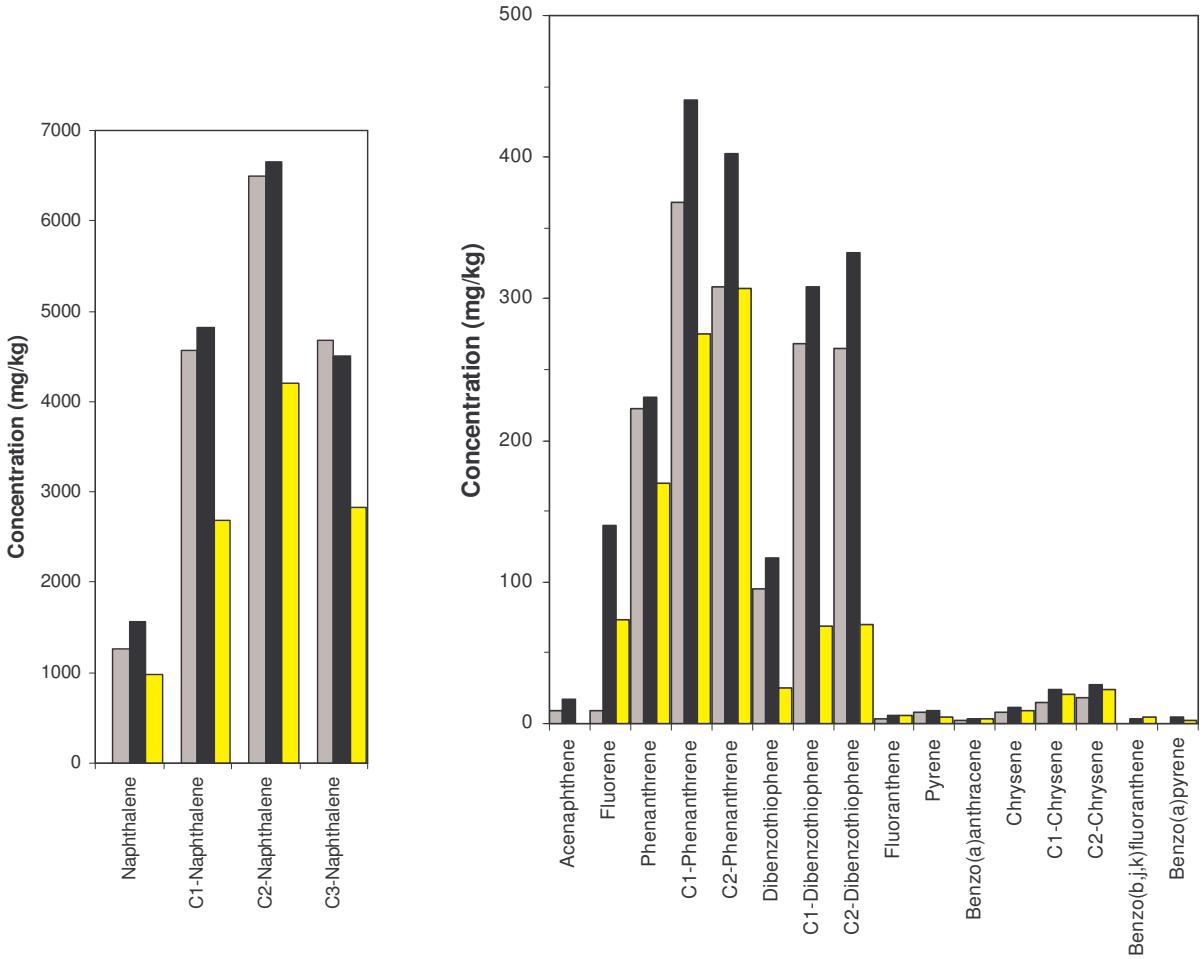


Figure 30. The mean concentration of C0-C3 naphthalenes (*left)* and 3 – 5 ring PAHs *(right*) in Goliat oil from BioSea I, BioSea II phase 1 and 2. No 6-ring PAH was recorded above the quantification limit in the Goliat oil. The grey bars show the concentration in 2004 Goliat/Real oil, black bars show 2007 Goliat/Real oil and the yellow bars show 2008 Goliat/Kobbe oil.

Comparison of the PAH concentration in the exposure tanks in the 2004, 2007 and 2008 shrimp larvae experiment

The lowest concentration tested in the 2007 experiment (0.015 mg/L) was repeated in the present experiment. The concentration of naphthalenes in the Kobbe batch of Goliat oil (tested in 2008) was approximately 50 % lower than in the Real batch used in BioSea I (2004) and in BioSea II (2007) phase 1. The results from the analysis of PAHs in water samples from the aquaria showed that the exposures had 50 % lower concentration of naphthalenes in the 0.015 mg/L oil treatment in 2008 than in 2007, indicating that the concentrations of oil were similar in the two experiments.

Figure 31 also show that although the concentration of PAH in the oil was the same in the 2004 and 2007 shrimp experiments the concentration in the exposure tanks were higher in 2004 (in the 0.015 mg/L oil exposure). This result indicates that the actual oil concentration was higher in the 2004 shrimp experiment than in the 2007 shrimp experiment.

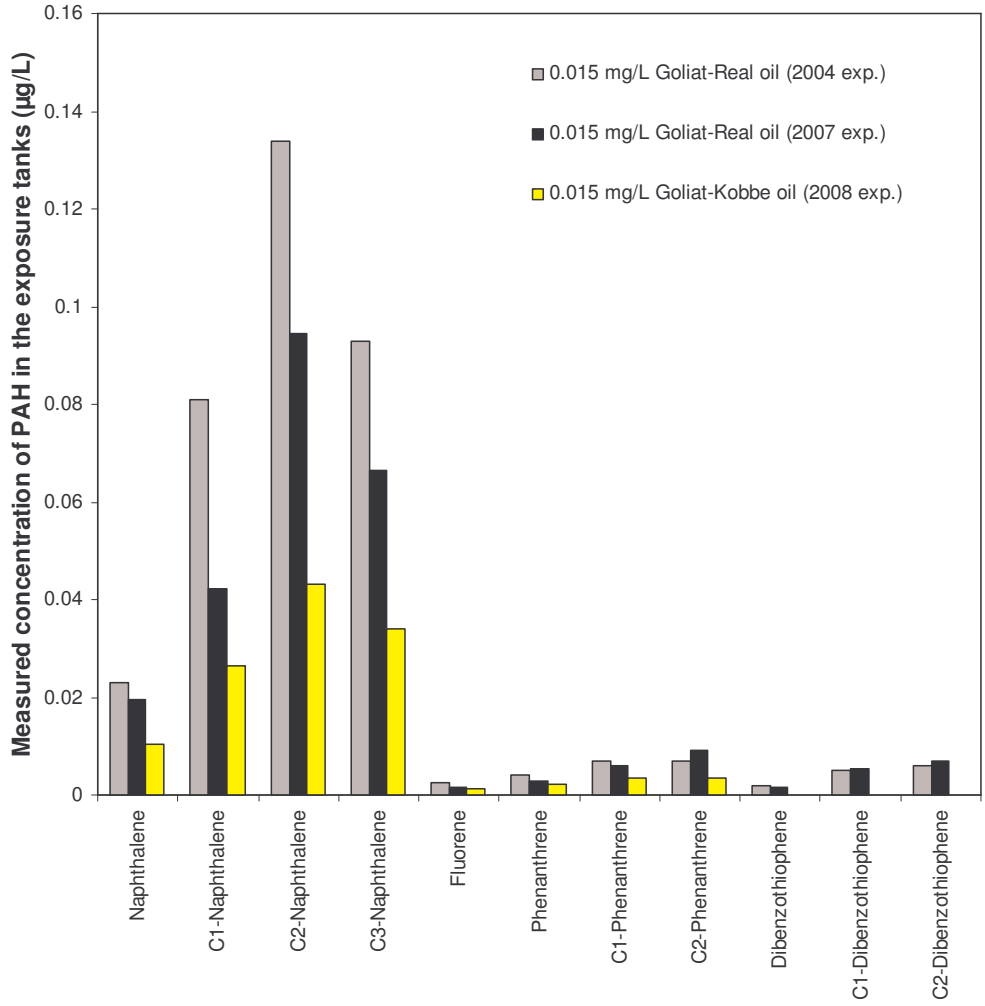


Figure 31. Comparison of the mean PAH concentration in the exposure tanks with 0.015 mg/L nominal oil in the 2004, 2007 and the 2008 shrimp larvae experiment in BioSea I and BioSea II JIPs.

Nominal[6] vs measured concentrations

Table 9 compare the relationship between nominal and measured PAH concentrations in the 2007 and 2008 shrimp larvae experiments. The boxes below show the formulas for calculating the nominal concentration of PAH in the exposure aquaria based on the measured concentration of PAH in the oil in the 2007 and 2008 shrimp larvae experiment. The values for the 2008 experiment were also given in Table 6 above, and details of the calculations are given in Appendix 13.

Table 9. The values in the table are the nominal concentration of PAH divided by the measured concentrations of PAH in the 2007 and 2008 shrimp larvae experiment. (See Appendix 13 for details).

	Nominal PAH conc. / Measured PAH conc. in the aquarium			
Type of oil:	Goliat-Kobbe 2008		Goliat-Real oil (20007)	
Nominal oil concentration:	0.010 mg/L	**0.015 mg/L**	**0.015 mg/L**	0.06 mg/L
	Nominal conc./ Measured conc.	Nominal conc./ Measured conc.	Nominal conc./ Measured conc.	Nominal conc./ Measured conc.
Naphthalene	2.0	**1.4**	**1.2**	1.0
C1-Naphthalene	2.1	**1.5**	**1.7**	1.0
C2-Naphthalene	1.7	**1.5**	**1.1**	0.8
C3-Naphthalene	1.4	**1.3**	**1.0**	0.8
Fluorene	0.8	**0.9**	**1.3**	1.0
Phenanthrene	1.3	**1.2**	**1.2**	1.0
C1-Phenanthrene	1.5	**1.2**	**1.1**	0.9

In the 2008 experiment more of C0, C2 and C3 naphthalene were lost than in 2007, but less C1-naphthalene and fluorene, and just as much phenanthrene was lost in 2008 as in 2007 (Table 9). The loss of more of the 2-rings in 2008 than in 2007 could be due to the fact that the oil used in 2008 was lighter (Figure 17). Based on the phenanthrene level the actual oil concentration in 0.015 mg/L was ca 20 % lower than the nominal both in 2007 and 2008 (0.012 mg/L). The actual concentration in 0.060 mg/L in the 2007 experiment was the same as the nominal, and the actual oil concentration 0.010 mg/L was 30 % lower than the nominal (0.007 mg/L).

Estimating actual oil concentrations in this way might not be accurate, but the approach can be used to compare the relative concentrations in the 2007 and 2008 experiment. Larsen (2004) commented on the inaccuracy of comparing measured PAH concentrations to the PAH concentration in the oil when the aim is to find the actual oil concentration in the exposure tank, and recommended analysis of THC in the aquaria

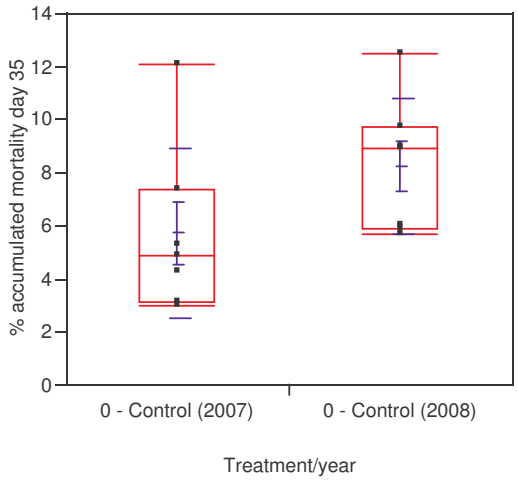
6 The *nominal* concentrations presented in Table 6 and 9 are **not** the same as the *predicted* concentrations in Table 3. The predicted concentrations were based on the measured PAH concentrations **in the aquaria in 2007**. That was done to achieve an oil concentration in the aquaria in 2008 that was as close to the actual oil concentration in the 0.015 mg/L treatment in 2007 as possible. The nominal PAH concentrations are calculated based on the measured PAH concentrations in the oil used in each experiment and the nominal dilution factors for the oil. This is done to get an indication of the actual oil concentrations.

and fluorescence of water samples from the aquaria (page 28, AM-2004/16). In the present experiment, however, the oil concentrations in the aquaria were below the detection limit for analysis of THC. Therefore we had to make an approximate prediction of PAH levels in the aquaria based on PAH concentrations in the oil to find the lowest oil concentration where it was possible to detect quantifiable concentrations of PAH in the pre-test.

Effects

Comparison of control mortality 2007 vs 2008

The mean accumulated mortality in the control group 35 days post-hatch was 2 % higher in 2008 than in 2007 (Figure 32). This may be explained by differences in the general condition of offspring in different years or genetic variability.



	Number of replicates	Mean	Std. Dev.	Std. Err. (Mean)	Median
0 - Control (2007)	7	5.72	3.17	1.20	4.9
0 - Control (2008)	7	8.24	2.52	0.95	8.9

Figure 32. The mortality in the control was slightly lower in 2007 than in 2008 (Wilcoxon, p = 0.047).

Comparison of variability (2007 vs 2008)

The variability in mortality between replicates in the oil exposed groups was higher in 2008 than in 2007. The mean accumulated mortality day 35 was 11% both years, but st.dev. was 3 in 2007 and 6 in 2008.

In the present experiment there was a slight increase in mortality in both oil exposed groups, but the increase was too small and the variability within groups too high to be statistically significant.

Comparison of effects on oil exposed shrimp larvae in 2007 and 2008

The LOEC for the 2007 oil was 0.015 mg/L (Taban *et al.* 2007). Because the Goliat/Kobbe oil tested in 2008 had a different chemical composition to the Goliat/Real oil tested in 2007, the results from the 2008 shrimp larvae experiment can not be used to determine a NOEC for the 2007 experiment.. The lower concentration of PAHs in the Goliat/Kobbe oil to the oil tested in 2007 from Goliat/Real may explain why there were less negative effects in the 2008 experiment at the same nominal oil concentration. The NOEC for the Goliat/Kobbe oil tested in the present experiment was 0.015 mg/L oil (Scenario 3 in Table 1).

In the BioSea I experiment with Goliat/Real in 2004, shrimp embryos were exposed for three months to 0.015 mg/L, 0.06 mg/L and 0.25 mg/L Goliat oil. After hatching, the larvae were kept in clean water and mortality and development time were studied. The lowest tested concentration was the LOEC for increased mortality of oil exposed shrimp larvae. The measured PAH concentrations (sum of the same compounds as in Table 10) were 0.34 µg/L, 1.13 µg/L and 4.91 µg/L PAH in the three treatments, respectively.

Based on these three BioSea experiments we can conclude that oil exposure of shrimp embryos and shrimp larvae both causes increased mortality. Results from 2007 showed a significant increase in mean accumulated mortality after only 18 days of exposure to 0.015 mg/L oil. We do not, however, know which compounds in the oil cause the effects on the shrimp larvae. It is possible that the PAHs influence the toxicity and if that is the case the lower toxicity observed in the 2008 experiment may be related to the lower concentration of PAHs in the Goliat/Kobbe oil.

Table 10. Nominal concentrations of oil, measured PAH concentrations (sum PAHs that were above detection limit in both experiments: C0-C3 naphthalenes, fluorene, C0-C2 phenanthrenes), net increase in mortality (mean accumulated mortality day 35 in exposed minus corresponding control mortality) and the conclusions from the 2007 and the 2008 experiments with shrimp larvae.

Nominal oil concentrations	Measured PAH concentrations	Net increase in mortality	Conclusion from 2007 exp. with Goliat/Real	Conclusion from 2008 exp. with Goliat/Kobbe
0.010 mg/L Goliat/Kobbe 2008	0.07 µg/L	3.3 %	Not tested	No significant effect
0.015 mg/L Goliat/Kobbe 2008	0.12 µg/L	2.4 %	Not tested	No significant effect = **NOEC**
0.015 mg/L Goliat/Real 2007	0.24 µg/L	4.9 %	Significant effect = **LOEC**	Not tested
0.060 mg/L Goliat/Real 2007	1.29 µg/L	25.0 %	Significant effect	Not tested

In the synthesis report these results will be compared to the results from the other experiments in BioSea II phase 2.

6 References

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Appendix 1 – 14: Background data & Research plan

Appendix 1. Flow of oil dispersion and seawater into each mixing flask.

	0.010 mg/L		0.015 mg/L	
	Water (3.8 L)	Oil (8.4 ml)	Water (4.1 L)	Oil (13.5 ml)
11-Mar	3.44	8.2	3.96	13.2
12-Mar	3.84	8.0	4.26	14.0
13-Mar	3.74	8.2	3.96	13.6
14-Mar	3.08	8.4	3.84	13.5
16-Mar	3.6	8.2	3.81	13.6
17-Mar	3.66	8.3	3.72	13.6
18-Mar	3.63	8.4	3.84	13.8
19-Mar	3.53	8.2	3.78	13.6
20-Mar	3.54	8.3	3.69	14
21-Mar	4.02	8.3	4.14	14
23-Mar	3.66	8.1	4.08	13.4
24-Mar	3.87	8.3	3.97	13.6
25-Mar	3.44	8.4	4.11	13.6
26-Mar	4.08	8.3	4.11	13.2
27-Mar	3.95	8.2	4.32	13.8
28-Mar	3.9	8.1	4.08	13.6
29-Mar	3.91	8.2	4.2	13.9
31-Mar	4.02	8.2	4.2	13.4
01-Apr	4.05	8.2	4.08	13.4
02-Apr	3.8	8.2	4.2	13.6
03-Apr	3.96	8.2	4.2	14
04-Apr	3.78	8.4	4.19	13.8
07-Apr	3.95	8.2	4.11	13.6
08-Apr	3.96	8.2	4.11	13.8
09-Apr	3.93	8.3	4.26	13.9
10-Apr	4.07	8.1	4.26	13.6
11-Apr	4.02	8.1	4.43	13.6
12-Apr	3.95	8	4.44	14
14-Apr	3.51	8.2	3.69	13.5
15-Apr	3.54	8.1	3.84	13.6
16-Apr	3.54	8.4	3.78	13.6
17-Apr	3.51	8.1	3.72	13.6
18-Apr	3.6	8.2	3.9	13.5
mean	3.76	8.2	4.04	13.7
st.dev	0.2	0.1	0.2	0.2

Appendix 2. Measured flow into each larvae aquarium/compartment

Flow ml/min

Treatment	Replicate	Compartment	1 week exposure	2 weeks exposure	5 weeks exposure
Control	1	L	120	120	120
Control	1	R	120	120	120
Control	2	L	100	110	120
Control	2	R	100	110	114
Control	3	L	125	130	134
Control	3	R	110	116	120
Control	4	L	120	130	124
Control	4	R	110	120	120
Control	5	L	125	130	124
Control	5	R	110	104	110
Control	6	L	120	116	126
Control	6	R	120	120	110
Control	7	L	120	118	104
Control	7	R	120	120	118
0.010 mg/L Goliat oil	1	L	96	84	80
0.010 mg/L Goliat oil	1	R	125	140	134
0.010 mg/L Goliat oil	2	L	90	140	136
0.010 mg/L Goliat oil	2	R	125	120	120
0.010 mg/L Goliat oil	3	L	140	164	120
0.010 mg/L Goliat oil	3	R	80	114	140
0.010 mg/L Goliat oil	4	L	110	120	120
0.010 mg/L Goliat oil	4	R	150	126	124
0.010 mg/L Goliat oil	5	L	120	124	120
0.010 mg/L Goliat oil	5	R	120	134	130
0.010 mg/L Goliat oil	6	L	90	140	146
0.010 mg/L Goliat oil	6	R	85	140	140
0.010 mg/L Goliat oil	7	L	125	130	160
0.010 mg/L Goliat oil	7	R	160	152	110
0.015 mg/L Goliat oil	1	L	124	122	126
0.015 mg/L Goliat oil	1	R	120	120	120
0.015 mg/L Goliat oil	2	L	140	144	150
0.015 mg/L Goliat oil	2	R	120	124	120
0.015 mg/L Goliat oil	3	L	118	120	120
0.015 mg/L Goliat oil	3	R	126	130	124
0.015 mg/L Goliat oil	4	L	140	124	160
0.015 mg/L Goliat oil	4	R	100	96	104
0.015 mg/L Goliat oil	5	L	130	134	104
0.015 mg/L Goliat oil	5	R	120	118	160
0.015 mg/L Goliat oil	6	L	160	108	160
0.015 mg/L Goliat oil	6	R	100	98	114
0.015 mg/L Goliat oil	7	L	90	100	86
0.015 mg/L Goliat oil	7	R	150	144	144

Appendix 3. Temperature and oxygen in each exposure aquarium.

Temperature (ºC)

Treatment	Replicate	1 week exposure	2 weeks exposure	3 weeks exposure	4 weeks exposure	5 weeks exposure
Control	1	5.0	4.9	5.2	5.3	5.1
Control	2	4.8	4.8	4.9	5.1	4.9
Control	3	4.8	4.6	4.8	5.0	4.7
Control	4	4.9	4.9	4.9	5.1	4.9
Control	5	4.8	4.8	4.9	5.1	4.9
Control	6	4.7	4.6	4.7	5.0	4.7
Control	7	4.9	4.9	4.9	5.1	5.0
0.010 mg/L Goliat oil	1	5.3	5.2	5.2	6.5	5.2
0.010 mg/L Goliat oil	2	4.9	4.9	5.1	5.2	5.1
0.010 mg/L Goliat oil	3	4.7	4.7	4.8	5.1	4.9
0.010 mg/L Goliat oil	4	4.9	4.9	5.0	5.2	5.0
0.010 mg/L Goliat oil	5	5.0	4.9	5.1	5.2	5.1
0.010 mg/L Goliat oil	6	5.2	5.1	4.9	5.1	4.9
0.010 mg/L Goliat oil	7	5.0	4.9	5.1	5.2	5.0
0.015 mg/L Goliat oil	1	5.0	5.0	5.1	5.3	5.1
0.015 mg/L Goliat oil	2	4.7	4.6	4.9	5.0	4.8
0.015 mg/L Goliat oil	3	5.1	4.7	4.8	5.0	4.9
0.015 mg/L Goliat oil	4	4.7	4.7	4.8	5.1	4.8
0.015 mg/L Goliat oil	5	5.0	4.9	5.0	5.3	5.1
0.015 mg/L Goliat oil	6	4.8	4.7	5.0	5.2	4.9
0.015 mg/L Goliat oil	7	5.0	4.9	5.1	5.3	5.1

Oxygen (%)

Treatment	Replicate	1 week exposure	2 weeks exposure	3 weeks exposure	4 weeks exposure	5 weeks exposure
Control	1	88	94	98	85	88
Control	2	85	94	97	92	87
Control	3	92	94	98	87	89
Control	4	85	92	98	88	89
Control	5	90	95	99	92	92
Control	6	87	95	98	88	89
Control	7	86	95	99	89	90
0.010 mg/L Goliat oil	1	88	95	99	89	91
0.010 mg/L Goliat oil	2	86	95	99	89	91
0.010 mg/L Goliat oil	3	87	94	99	90	90
0.010 mg/L Goliat oil	4	87	96	96	90	88
0.010 mg/L Goliat oil	5	89	96	96	90	90
0.010 mg/L Goliat oil	6	88	96	99	90	91
0.010 mg/L Goliat oil	7	88	96	99	89	90
0.015 mg/L Goliat oil	1	88	96	100	85	94
0.015 mg/L Goliat oil	2	87	96	99	90	90
0.015 mg/L Goliat oil	3	89	96	100	89	91
0.015 mg/L Goliat oil	4	87	96	98	87	89
0.015 mg/L Goliat oil	5	87	94	99	90	89
0.015 mg/L Goliat oil	6	87	95	100	86	90
0.015 mg/L Goliat oil	7	86	96	99	88	89

Oxygen (mg/L)

Treatment	Replicate	1 week exposure	2 weeks exposure	3 weeks exposure	4 weeks exposure	5 weeks exposure
Control	1	10.8	11.6	12.2	10.5	11.1
Control	2	10.8	11.8	12.1	11.5	11.0
Control	3	11.7	11.7	12.3	10.9	11.2
Control	4	10.7	11.4	12.3	11.0	11.2
Control	5	11.7	11.7	12.4	11.4	11.6
Control	6	11.1	11.8	12.4	11.1	11.3
Control	7	10.9	11.7	12.3	11.1	11.3
0.010 mg/L Goliat oil	1	11.1	11.7	12.3	10.7	11.4
0.010 mg/L Goliat oil	2	10.9	11.8	12.3	11.0	11.4
0.010 mg/L Goliat oil	3	11.1	11.8	12.3	11.3	11.4
0.010 mg/L Goliat oil	4	11.0	11.8	11.9	11.2	11.0
0.010 mg/L Goliat oil	5	11.2	11.9	12.0	11.2	11.3
0.010 mg/L Goliat oil	6	11.1	11.7	12.4	11.3	11.5
0.010 mg/L Goliat oil	7	11.1	12.0	12.4	11.0	11.4
0.015 mg/L Goliat oil	1	11.1	11.8	12.5	10.6	11.8
0.015 mg/L Goliat oil	2	11.1	12.0	12.4	11.2	11.5
0.015 mg/L Goliat oil	3	11.3	12.0	12.5	11.1	11.5
0.015 mg/L Goliat oil	4	11.1	12.0	12.3	10.9	11.2
0.015 mg/L Goliat oil	5	10.9	11.7	12.4	11.1	11.1
0.015 mg/L Goliat oil	6	11.1	11.8	12.5	10.6	11.4
0.015 mg/L Goliat oil	7	10.9	11.9	12.3	10.9	11.1

Appendix 4. Light conditions (lux) above the aquaria.

Treatment	Replicate	Lux
Control	1	36
Control	2	25
Control	3	28
Control	4	21
Control	5	24
Control	6	16
Control	7	20
0.010 mg/L Goliat oil	1	38
0.010 mg/L Goliat oil	2	41
0.010 mg/L Goliat oil	3	30
0.010 mg/L Goliat oil	4	28
0.010 mg/L Goliat oil	5	9
0.010 mg/L Goliat oil	6	20
0.010 mg/L Goliat oil	7	15
0.015 mg/L Goliat oil	1	31
0.015 mg/L Goliat oil	2	23
0.015 mg/L Goliat oil	3	19
0.015 mg/L Goliat oil	4	22
0.015 mg/L Goliat oil	5	38
0.015 mg/L Goliat oil	6	9
0.015 mg/L Goliat oil	7	4
	mean	24
	st.dev.	10

Appendix 5. Mortality of shrimp larvae in the **control.** From day 1 – 10 the larvae are mainly on stage I. In the period from day 10 – 13 there are both stage I and stage II larvae present in the left compartment of each aquarium. From day 13-20 there are mainly stage II larvae in the left compartment. In the period from day 20 – ca 25 all larvae that had developed to stage III were transferred from the left to the right compartment of the aquarium each day. At the end of the experiment most of the larvae had developed to stage IV larvae.

Treatment:	Control		Control		Control		Control		Control		Control	
Started (date):	11 March 2008		11 March 2008		11 March 2008		11 March 2008		12 March 2008		12 March 2008	
Replicate:	1		2		3		4		5		6	
Compartment:	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV
Total no. at start:	298		305		299		299		302		264	
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0		0		0		1		0		0	
2	0		0		0		0		0		0	
3	0		0		0		0		0		0	
4	1		1		2		0		1		1	
5	0		1		0		1		1		1	
6	2		2		0		3		1		2	
7	0		0		1		1		0		1	
8	1		0		0		0		0		3	
9	0		0		4		1		0		3	
10	0		0		0		0		0		0	
11	0		0		0		0		0		0	
12	0		0		0		1		0		0	
13	2		1		0		0		0		0	
14	0		2		0		3		0		1	
15	0		0		0		0		2		0	
16	0		0		0		5		1		4	
17	1		2		0		3		0	0	3	0
18	1	0	0	0	1	0	1	0	1	0	2	0
19	0	0	0	0	1	0	2	0	0	0	1	0
20	0	0	1	0	1	0	1	0	7	0	3	0
21	0	0	2	0	0	0	0	0	1	0	2	0
22	2	0	1	0	0	0	0	0	1	0	1	0
23	1	0	3	0	0	0	0	0	0	1	3	0
24	0	0	0	0	1	0	1	1	0	1	0	0
25	0	0	0	1		1		1		0	0	1
26	1	0	0	0		0		0		2	0	0
27		0	0	0		0		0		1	0	0
28		1	0	0		0		0		0	0	0
29		0	0	0		1		0		1		0
30		2	0	0		0		1		0		0
31		0	0	0		0		0		2		0
32		0	0	0		1		0		0		0
33		0	0	0		0		0		1		0
34		0	0	0	0	0		2		1		1
35		3	0	1	0	3		0		1		0
dag 0-35	12	6	16	2	11	6	24	5	16	11	31	2

Appendix 6. Mortality of shrimp larvae in the **0.010 mg/L Goliat/Kobbe exposure.** From day 1 – 10 the larvae are mainly on stage I. In the period from day 10 – 13 there are both stage I and stage II larvae present in the left compartment of each aquarium. From day 13-20 there are mainly stage II larvae in the left compartment. In the period from day 20 – ca 25 all larvae that had developed to stage III were transferred from the left to the right compartment each day. At the end of the experiment most of the larvae had developed to stage IV larvae.

Appendix 6

Treatment:	0.010 mg/L		0.010 mg/L		0.010 mg/L		0.010 mg/L		0.010 mg/L		0.010 mg/L	
Started (date):	11 March 2008		11 March 2008		11 March 2008		11 March 2008		12 March 2008		12 March 2008	
Replicate:	1		2		3		4		5		6	
Compartment:	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV
Total no. at start:	302		300		299		308		265		283	
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0		0		0		0		0		0	
2	0		1		0		0		0		0	
3	0		0		0		0		2		3	
4	0		1		1		1		1		2	
5	1		0		0		3		0		1	
6	0		1		3		0		0		4	
7	1		0		1		4		0		1	
8	2		1		1		0		1		0	
9	0		0		1		0		0		0	
10	0		0		0		4		0		1	
11	2		0		1		0		1		0	
12	0		0		1		3		4		0	
13	0		0		1		1		3		0	
14	0		0		1		0		7		0	
15	0		0		1		2		5		0	
16	0		2		0		0		11		1	
17	1		2		5		7		5	0	2	0
18	2	0	0	0	2	0	6	0	5	0	0	0
19	1	0	1	0	2	0	2	0	3	0	2	0
20	2	0	2	0	2	0	1	0	3	0	3	0
21	6	0	1	0	0	0	0	0	0	0	0	0
22	0	0	0	0	1	0	0	0	2	0	0	0
23	0	0	1	0	2	0	1	1	2	0	0	0
24	0	1	0	1	1	2	0	0	0	1	0	0
25	0	1		1	0	0	0	2	0	0	1	0
26	2	0		0	2	0		1	0	1		0
27	0	0		0		0		2	0	0		1
28	0	0		0		0		0	1	1		0
29	0	0		0		1		0	1	2		0
30	0	0		1		1		0	0	0		0
31	0	0		0		0		0		0		1
32	0	0		1		0		4		0		0
33	0	0		0		1		0		0		0
34	0	0		0		0		0		0		1
35	0	1		1		0		2		0		1
dag 0-35	20	3	13	5	29	5	35	12	57	5	21	4

Appendix 7. Mortality of shrimp larvae in the **0.015 mg/L Goliat/Kobbe exposure.** From day 1 – 10 the larvae are mainly on stage I. In the period from day 10 – 13 there are both stage I and stage II larvae present in the left compartment of each aquarium. From day 13-20 there are mainly stage II larvae in the left compartment. In the period from day 20 – ca 25 all larvae that had developed to stage III were transferred from the left to the right compartment each day. At the end of the experiment most of the larvae had developed to stage IV larvae.

Treatment:	0.015 mg/L		0.015 mg/L		0.015 mg/L		0.015 mg/L		0.015 mg/L		0.015 mg/L	
Started (date):	11 March 2008		11 March 2008		11 March 2008		11 March 2008		12 March 2008		12 March 2008	
Replicate:	1		2		3		4		5		6	
Compartment:	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV	L: Stage I/II	R: Stage III/IV
Total no. at start:	293		304		300		309		288		299	
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0		0		0		0		0		0	
2	0		0		0		0		1		0	
3	0		0		0		0		0		1	
4	0		0		0		0		0		0	
5	0		0		1		0		0		0	
6	0		0		2		1		0		6	
7	0		0		2		2		2		1	
8	1		0		0		1		0		2	
9	3		1		4		0		1		4	
10	0		0		1		1		0		4	
11	1		2		0		0		1		1	
12	0		0		1		0		0		0	
13	0		0		3		1		0		1	
14	0		0		1		1		2		0	
15	3		2		2		4		0		0	
16	2		0		0		0		0		0	
17	1		3		11		1		1	0	1	0
18	3	0	4	0	6	0	2	0	0	0	1	0
19	3	0	11	0	4	0	1	0	0	0	0	0
20	1	0	2	0	3	0	0	0	0	0	0	0
21	1	0	1	0	1	0	0	0	0	0	2	0
22	1	0	2	0	2	0	1	0	0	0	2	0
23	1	0	1	2	1	1	2	0	0	0	1	1
24	1	0	0	2	3	0	1	0	0	0	1	0
25	1	1	1	0	1	1	1	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	1	0
27	1	1	0	1		2	1	0	0	1	0	0
28		0	0	2		1	0	1	0	0	0	0
29		0	0	1		0	0	0	0	1	1	2
30		1	1	0		1	1	2	0	0	0	1
31		0	0	1		1	1	0	0	0	0	0
32		0	1	1		3	1	0	0	1	0	0
33		0		0		0	0	1	0	0	0	0
34		0		1		1	0	0	0	0	0	0
35		1		0		2	1	0	0	0	0	0
dag 0-35	24	4	32	11	49	13	25	4	8	3	30	4

Appendix 8. Mean percent accumulated mortality with increasing exposure time for shrimp larvae in the control group and exposed to 0.010 and 0.015 mg/L oil.

Exposure time (days)	Control	0.010 mg/L Goliat/Kobbe	0.015 mg/L Goliat/Kobbe
0	0.0	0.0	0.0
1	0.1	0.1	0.0
2	0.1	0.1	0.0
3	0.2	0.5	0.2
4	0.5	0.9	0.3
5	0.9	1.1	0.3
6	1.5	1.5	0.8
7	1.6	1.9	1.2
8	1.9	2.2	1.4
9	2.3	2.2	2.1
10	2.3	2.4	2.4
11	2.3	2.6	2.6
12	2.4	3.0	2.7
13	2.5	3.3	2.9
14	2.9	3.8	3.1
15	3.0	4.2	3.8
16	3.5	4.9	3.9
17	4.0	6.0	4.8
18	4.3	6.9	5.6
19	4.5	7.5	6.5
20	5.2	8.2	6.8
21	5.5	8.5	7.0
22	5.8	8.7	7.4
23	6.3	9.0	7.9
24	6.5	9.4	8.3
25	6.7	9.7	8.6
26	6.9	10.1	8.7
27	6.9	10.3	9.0
28	7.0	10.4	9.2
29	7.1	10.7	9.4
30	7.2	10.8	9.8
31	7.3	10.8	10.0
32	7.3	11.1	10.3
33	7.4	11.2	10.3
34	7.6	11.2	10.4
35	8.3	11.5	10.7

Appendix 9. Percent shrimp larvae on stage III with increasing exposure time. Exposure time (days) = number of days after hatching. Data used in Figure 23.

Exposure time (days)	Control	Control	Control	Control	Control	Control	Control	mean control
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
21	10	20	1	1	54	0	4	13
22	77	92	57	66	98	20	59	67
23	98	99	97	99	100	84	98	96
24	100	99	100	100	100	97	100	99
25	100	99	100	100	100	99	100	100
26	100	100	100	100	100	99	100	100
27	100	100	100	100	100	99	100	100
28	100	100	100	100	100	100	100	100

Exposure time (days)	0.010 mg/L	0.010 mg/L	0.010 mg/L	0.010 mg/L	0.010 mg/L	0.010 mg/L	0.010 mg/L	mean 0.010 mg/L
19	0	0	0	0	0	0	0	0
20	22	1	0	0	25	18	0	9
21	84	49	1	45	92	81	10	52
22	98	97	26	92	99	96	77	84
23	99	100	81	99	99	100	97	96
24	99	100	96	100	100	100	100	99
25	99	100	99	100	100	100	100	100
26	100	100	100	100	100	100	100	100
27	100	100	100	100	100	100	100	100
28	100	100	100	100	100	100	100	100

Exposure time (days)	0.015 mg/L	0.015 mg/L	0.015 mg/L	0.015 mg/L	0.015 mg/L	0.015 mg/L	0.015 mg/L	mean 0.015 mg/L
19	0	0	0	0	0	0	0	0
20	0	0	0	0	1	0	2	0
21	22	0	2	0	55	20	66	24
22	86	28	53	17	93	64	99	63
23	97	75	95	78	99	84	100	90
24	99	99	98	96	100	97	100	98
25	99	100	100	100	100	100	100	100
26	99	100	100	100	100	100	100	100
27	100	100	100	100	100	100	100	100
28	100	100	100	100	100	100	100	100

Appendix 10. Percent shrimp larvae on stage IV at the end of the experiment (35 days post-hatch).

	% stage IV of living day 35	% stage IV of total at start
0 - Control	99	86
0 - Control	98	92
0 - Control	99	93
0 - Control	100	89
0 - Control	97	88
0 - Control	95	83
0 - Control	96	85
0.010 mg/L Goliat/Kobbe	95	86
0.010 mg/L Goliat/Kobbe	99	93
0.010 mg/L Goliat/Kobbe	98	87
0.010 mg/L Goliat/Kobbe	100	84
0.010 mg/L Goliat/Kobbe	99	75
0.010 mg/L Goliat/Kobbe	97	88
0.010 mg/L Goliat/Kobbe	99	90
0.015 mg/L Goliat/Kobbe	94	85
0.015 mg/L Goliat/Kobbe	99	83
0.015 mg/L Goliat/Kobbe	98	74
0.015 mg/L Goliat/Kobbe	99	88
0.015 mg/L Goliat/Kobbe	92	89
0.015 mg/L Goliat/Kobbe	95	81
0.015 mg/L Goliat/Kobbe	99	93

Appendix 11. Measured PAH concentration in water samples from the exposure aquaria

Nominal oil-concentration: 0.010 mg/L Goliat-Kobbe oil

µg/L	Week 1	Week 2	Week 3	Week 4	Week 5
Naphthalene	0.007	0.004	0.006	0.004	0.003
C1-Naphthalene	0.015	0.012	0.013	0.013	0.012
C2-Naphthalene	0.030	0.023	0.023	0.024	0.021
C3-Naphthalene	0.024	0.018	0.020	0.020	0.016
Fluorene	0.001				
Phenanthrene	0.002		0.001	0.001	0.001
C1-Phen/Anthr	0.002	0.002	0.002	0.002	0.002
C2-Phen/Anthr					
sum PAH	**0.08**	**0.06**	**0.07**	**0.06**	**0.06**

Nominal oil-concentration: 0.015 mg/L Goliat-Kobbe oil

µg/L	Week 1	Week 2	Week 3	Week 4	Week 5
Naphthalene	0.012	0.011	0.011	0.009	0.009
C1-Naphthalene	0.025	0.026	0.029	0.027	0.025
C2-Naphthalene	0.044	0.043	0.049	0.040	0.040
C3-Naphthalene	0.038	0.033	0.035	0.031	0.033
Fluorene	0.001		0.001	0.001	0.001
Phenanthrene	0.002		0.002	0.002	0.002
C1-Phen/Anthr	0.003	0.004	0.003	0.003	0.004
C2-Phen/Anthr	0.004	0.004	0.003	0.003	0.003
sum PAH	**0.13**	**0.12**	**0.13**	**0.12**	**0.12**

Appendix 12. Predicted PAH concentrations used in the pre-test (see Table 3).

PAH compound	mg PAH/kg Goliat-Real oil Tested in 2007	mg PAH/kg Goliat-Kobbe oil Tested in 2008	**F** = PAH in Goliat-Real / PAH in Goliat-Kobbe	Mean measured PAH conc. in the aquaria with 0.015 mg/L Goliat Real oil in 2007 µg PAH/L **A**	Predicted PAH conc. in 0.015 mg/L Goliat-Kobbe oil **A/F**	Predicted PAH conc. in 0.010 mg/L Goliat-Kobbe oil (**A/F**) - (33%)
Naphthalene	1558	985	1.6	0.026	0.016	0.011
C1-Naphthalene	4815	2694	1.8	0.051	0.029	0.019
C2-Naphthalene	6655	4196	1.6	0.095	0.060	0.040
C3-Naphthalene	4509	2834	1.6	0.066	0.042	0.028
Phenanthrene	230	170	1.4	0.003	0.002	0.001
C1-Phen/Anthr	440	275	1.6	0.006	0.004	0.003
C2-Phen/Anthr	403	307	1.3	0.009	0.007	0.005
Dibenzothiophene	117	25	4.7	0.002	0.000	0.000
C1-Dibenzothiophene	308	69	4.5	0.005	0.001	0.001
C2-Dibenzothiophene	333	70	4.8	0.007	0.001	0.001

Appendix 13. Calculation of nominal PAH concentrations based on the PAH concentration in the oil (see Table 6 and 9).

2008	µg PAH/kg Goliat-Kobbe oil	Nominal PAH conc. in 0.010 mg/L Goliat-Kobbe oil	Nominal PAH conc. in 0.015 mg/L Goliat-Kobbe oil	Measued PAH conc. in 0.010 mg/L Goliat-Kobbe oil	Measured PAH conc. in 0.015 mg/L Goliat-Kobbe oil	Nominal PAH conc. / Measured PAH conc.	
						0.010 mg/L Goliat-Kobbe	0.015 mg/L Goliat-Kobbe
Naphthalene	985000	0.010	0.015	0.005	0.010	2.0	1.4
C1-Naphthalene	2694000	0.027	0.040	0.013	0.027	2.1	1.5
C2-Naphthalene	4196000	0.042	0.063	0.024	0.043	1.7	1.5
C3-Naphthalene	2834000	0.028	0.043	0.020	0.034	1.4	1.3
Fluorene	73000	0.001	0.001	0.001	0.001	0.8	0.9
Phenanthrene	170000	0.002	0.003	0.001	0.002	1.3	1.2
C1-Phen/Anthr	275000	0.003	0.004	0.002	0.003	1.5	1.2
2007	µg PAH/kg Goliat-Real oil	Nominal PAH conc. in 0.015 mg/L Goliat-Real oil	Nominal PAH conc. in 0.060 mg/L Goliat-Real oil	Measured PAH conc. in 0.015 mg/L Goliat-Real oil	Measured PAH conc. in 0.060 mg/L Goliat-Real oil	Nominal PAH conc. / Measured PAH conc.	
						0.015 mg/L Goliat-Real	0.060 mg/L Goliat-Real
Naphthalene	1557846	0.023	0.093	0.020	0.091	1.2	1.0
C1-Naphthalene	4815050	0.072	0.289	0.042	0.285	1.7	1.0
C2-Naphthalene	6655316	0.100	0.399	0.095	0.475	1.1	0.8
C3-Naphthalene	4509236	0.068	0.271	0.066	0.354	1.0	0.8
Fluorene	140401	0.002	0.008	0.002	0.008	1.3	1.0
Phenanthrene	229994	0.003	0.014	0.003	0.014	1.2	1.0
C1-Phen/Anthr	439666	0.007	0.026	0.006	0.029	1.1	0.9

Appendix 14. Scope of work / Research plan

1. Identity	
Biosea II JIP shrimp (*Pandalus borealis*) larvae in Goliat dispersed crude oil	
2. Purpose, background	
Study biological effects in shrimp larvae exposed to the following nominal concentrations of Goliat oil: 0.010 and 0.015 mg/L oil. The purpose is to determine a NOEC for the oil used, - the different scenarios anticipated and what we can conclude are outlined in more detail below (**14. Research design**).	
3. Customers	
Total, Eni	
4. Main test laboratory	
IRIS & Akvamiljø Mekjarvik 12 4070 Randaberg	
5. Research facility (for sub task):	
6. Research participants	**Task**
Renée K. Bechmann	Experiment leader, planning and participating in rigging and running, reporting
Ingrid C. Taban	Planning
Anna Ingvarsdottir, Brit Fjone Godal, Marianne Nilsen, Anne Helene Tandberg	Rigging and running the experiment including determination of mortality and stage
7. Participants in part of the experiment	**Task**
Helge Knutsen	Rigging the experiment
Rolf Sundt	Getting shrimp larvae, Running the CFS
Atle Nævdal	PAH analysis (SPE water)
8. Planned start of experiment	March 2008 (week depends on when the larvae hatch).
9. Planned end of experiment	April/May 2008
10. Background	
Test biological responses/effects to dispersed Goliat oil in the larvae of the northern shrimp (*Pandalus borealis*), a relevant invertebrate species in the Barents Sea.	

11. Materials and general treatment (excluding the exposures)	
Species: Stock/Source, origin: Veterinary attest:	* Northern shrimp *Pandalus borealis* * Local stock. * Adult shrimps will be collected locally by trawling using a local fisherman. Collection of shrimps will be done in February 2008..

(not relevant) Received date: Storage/ Acclimatization: Sex/Weight/Size/age: Feeding/Number:	* Adult female shrimps will be transferred to ~500L tanks with flow through seawater at 5°C for a minimum of two weeks of acclimatization, prior to transfer to hatching chambers held at the same temperature. * Large/larger females will be used. The exposure will be carried out using newly hatched shrimp larvae. * Adult shrimps will be fed raw fish twice a week during this period. The larvae will be fed once a day with newly hatched *Artemia salina* nauplii. * ~ 20-60 adult female shrimps (no. depends on how many larvae that are produced from each individual and on the hatching pattern) * ~ 6300 newly hatched shrimp larvae.

12. Test group overview (including exposure conditions)

The adult shrimps will be kept in clean water. Only the shrimp larvae will be used in the experiment. Total and Eni have decided to expose shrimp larvae to two concentrations of a new batch of Goliat oil (in addition to a control).

Chemical analysis of the PAH content in the new batch of Goliat oil was done in week 6. Results are shown in attachment 3. The new oil has a lower concentration of PAH than the oil used in the 2007 experiment (analysed in 2006). The total PAH content is about 30 % lower in the new oil sample. The 2007 oil had approximately twice as much naphthalenes, fluorene, C1-phenanthrene and pyrene, and 4-5 times as much DBTs per gram oil as the 2008 oil. The new oil contains much more of the lighter oil fractions, e.g. n-alkanes C9-C14 as the chromatogram shows. The nC17/Pristane ratio is also different.

0.015 mg/L oil: We will repeat the lowest concentration tested in the 2007 experiment (i.e. same mg/L of oil). In the 0.015 mg/L treatment in the 2007 experiment only C0-C3 naphthalenes were above the detection limit (SPE extraction) (see attachment 2). We will measure PAHs in the water in the pre–test and in the experiment. If the concentrations of naphthalenes are approximately 50% lower than in 2007 the amount of oil is similar to the levels found in the 2007 experiment (see attachment 3).

0.010 mg/L oil: In addition we will test an oil concentration that is as low as it is chemically possible to detect PAHs in the water. As the levels of naphthalenes are about 50% compared to the test preformed by Steinar Sanni (attachment 1) the results from this test is less relevant. Our aim will therefore be to pump 33 % less 5 mg/L oil dispersion into the mixing flask for this treatment.

The two chosen nominal concentrations are so close that we may not measure significantly different concentrations of PAH in the two oil treatments. The results from analysis of PAH in water samples from treatments with the same nominal oil concentration in BioSea I and II have shown the actual concentrations vary by a factor of nearly 2 (see figures in synthesis report from 2007). Some of these differences may be explained by the type of test animal (bivalve versus shrimp and fish), but other differences in experimental design is also important (e.g. length, thickness, splits and bends of tubing, water velocity etc.). This is one reason why we often space our concentrations more (by a factor of 4 in BioSea I and in the 2007 shrimp larvae experiment).

Group 1	Replicate 1-7	Control
Group 2	Replicate 1-7	0.010 mg/l Goliat oil in seawater (nominal)
Group 3	Replicate 1-7	0.015 mg/l Goliat oil in seawater (nominal)

13. Details of exposures components	
Group 2 and 3	New batch of Goliat dispersed crude oil. The continuous flow system (CFS) will be used to create the dispersion of crude oil in seawater. The 5 mg/L oil dispersion will be pumped into two mixing flasks and diluted to 0.010 mg/L and 0.015 mg/L. Each mixing flask has an overflow and 7 outlets – one for each replicate aquarium.

14. Research design (chronological procedure)

January 2008: Planning and rigging

February:

Trawling and transport of adult shrimps to Akvamiljø. Acclimatisation. Female shrimps transferred to hatching chambers.

Chemical analysis of the PAH content in the new batch of Goliat oil was done in week 6. Results are shown in attachment 3.

Analysis of PAH concentration (SPE extraction) in water samples from the exposure aquaria will be done before we start the experiment. This will be done in mid February to be ready to start the experiment in the beginning of March if necessary.

We will analyze PAH concentration (SPE extraction, and in addition one set of samples using liquid – liquid extraction for comparison) in one water sample from each test concentration before we start the experiment. This pre-test is necessary to reduce the risk that the concentration of PAH in the exposure aquaria is above the detection limit.

March: The experiment will most likely be started in March. The experiment will be run with the shrimp larvae. The exact timing of the experiment will depend on the hatching of the eggs. In the 2007 Biosea II experiments with shrimp larvae, the experiment was started 14th of March.

March-May: 5 weeks exposure of the shrimp larvae will be done in this period.

The adult shrimps will be collected in February and acclimatized for at least 2 weeks before being transferred to hatching aquaria (19 L aquaria with 2 compartments). The female shrimps will be placed in separate hatching chambers in order to collect larvae individually from each female. Furthermore, the day of hatching of the larvae from each female will be known. Hatching occurred over a 1-1.5 month period in the Goliat exposure in Biosea I. The different treatments and replicates will therefore start at different times, depending on hatching. It is impossible to predict precisely when the larvae are going to hatch from each individual female. Furthermore, larvae from one female do not all hatch within a day. In Biosea I and II (phase 1), most larvae from one individual hatched over a week long period. The larvae will be exposed for 5 weeks. Because the different replicates from each treatment will be started with a time lag depending on the hatching pattern of the females, the experiment will be run for a total of 6-7

weeks.

~12 hatching aquaria will be set up (each hatching aquaria will contain two female shrimps). The aquaria will be equipped with plankton net at the outlet to prevent the larvae from being lost. After hatching, the larvae will be kept in the same type of aquaria (19 L, two compartments) in groups of ~300. In the 2007 experiment we were not able to determine the stage of dead larvae. In the 2008 experiment we plan to use two compartment aquaria. First all the larvae are kept in one compartment. When the larvae develop into stage 3 larvae they will be transferred to the other compartment each day when stage in the period when stage is determined. This change in experimental design will make it possible to indicate whether the dead larvae have reached stage 3 or not (assuming that any dead larvae found in the stage 2 compartments of the aquaria has not changed stage to stage 3 within the last 24h).

The larvae in each larvae aquarium will be a mixture of the offspring from 2-3 mothers, depending on how many that hatches. Only larvae that appear to be fit will be used in the experiment. Larvae that are not swimming or are damaged/deformed will not be used in the experiment. The larvae will be kept at low light conditions (as in the 2007 BioSea II shrimp larvae experiment).

The Goliat oil used in this present experiment is different from the Goliat oil used in BioSea I and Biosea II (phase I) with shrimp larvae (see attachment 3). The toxicity of oil depends on the chemical composition and physical characteristics of the oil; hence we can not assume that we will find the same effects of the new batch of Goliat oil as for the oil tested in 2007. This is especially important when the aim is to test concentrations close to the expected NOEC. If the new batch of oil gives more effects than the oil tested in 2007 we may get effects in both the chosen concentrations in the present experiment. If the new oil is less toxic than the 2007 oil we may not see any effect in any of the chosen concentrations. In addition, one also has to anticipate variability in the general condition of offspring in different years.

The results we get in the 2008 experiment can not be used to determine a NOEC for the 2007 oil. The LOEC for the 2007 oil will be 0.015 mg/L.

The table below show results from the 2007 shrimp larvae experiment and 3 possible scenarios for 2008. Red boxes: significant effect. Green boxes: no significant effect. Grey boxes: not tested.

Nominal oil concentrations	Results from 2007	2008 Scenario 1	2008 Scenario 2	2008 Scenario 3
0.010 mg/L Goliat 2008				
0.015 mg/L Goliat 2007				
0.015 mg/L Goliat 2008				
0.060 mg/L Goliat 2007				

Scenario 1: LOEC for oil 2008 = 0.010 mg/L. We can not set a NOEC for oil 2008.
Scenario 2: LOEC for oil 2008 = 0.015 mg/L. NOEC for oil 2008 = 0.010 mg/L.
Scenario 3: NOEC for oil 2008 = 0.015 mg/L. We can not set a LOEC for oil 2008.

15. Test system and conditions (dose/administration)	
Test system: Exposure unit:	Pilot hall/CFS 21 (7x3) small aquaria (25x25x30 cm, volume 19 L, two

	compartments) will be used for the larvae. Each larvae aquarium will have a flow-through water supply rate of about ~150 ml per min into each compartment. The oil dispersion will be made by injection of oil in the flow of seawater using a precision pump. The pump delivers a constant flow of water into the lower part of a piston cell and oil is pushed out on the other side. To control that the flow of oil is constant throughout the experiment, the bottle of water will be placed on a balance and the weight of the bottle will be recorded daily. The 5 mg/L oil dispersion coming from the CFS will then be diluted further with seawater in two mixing flasks to obtain the two desired nominal concentrations (0.010 and 0.015 mg/L oil). The flow of sea water and dispersed Goliat oil into the two mixing flasks will be measured every weekday. The flow of test solution into each aquaria will be measured every second week.
Doses/concentrations: Exposure duration/	Control, 0.010 & 0.015 mg/l (nominal values, see **12** above).
No Depuration	5 weeks exposure of each replicate batch of shrimp larvae, no depuration.
16. Measurements / observations (Procedure or reference to SOP).	
Type of analysis	**Temperature** • Weekly temperature measurements in each larvae aquarium. • Daily measurements at one place in the water stream will be registered daily **Oxygen** • Weekly oxygen measurements in each larvae aquarium. • Salinity will be measured weekly at one place in the water stream. **Light** will be measured once during the experiment, in air, just above the water surface. **Survival**. Dead shrimp larvae will be removed from each larvae aquarium daily. Based on experience from BioSea I, an average accumulated mortality in the control group is expected to be around 25 % after 25 days. However, one also has to anticipate variability in the general condition of offspring in different years. In the 2007 shrimp larvae experiment accumulated mean mortality in the control was 6% after 35 days. The control mortality may be higher in 2008 than in 2007. The higher mortality observed in the BioSea I shrimp embryo exposure may have been due to the longer time the shrimps were kept in the lab, but we can not exclude the possibility that the quality of embryo/larvae differ between years (like for fish larvae). **Developmental stage of larvae.** When stage 2 larvae start to change to stage 3, the relative proportions of stage 2 and 3 will be determined on all larvae from each aquarium. Determination of stage will continue until the majority of larvae have reached stage 3. The majority of the larvae are expected to change stage approximately between day 21 and 30 (see Figure 18 in the 2007 shrimp larvae report). This will allow us to calculate percentage of larvae that have

	reached stage 3 with time. We will do the same type of data treatment as in the 2007 shrimp larvae report. Determination of stage can indicate whether development is delayed due to the oil exposure. Finally, at the last day of the experiment (day 35) the relative proportions of larvae at stage 3 and 4 will be determined (like in the 2007 experiment). Differences in the percentage of larvae reaching stage 4 within 35 days may indicate effects on development.
Responsible lab	IRIS & Akvamiljø To secure optimal output of study material, IRIS & Akvamiljø will consider an early termination of an exposure in the case of high sudden or accumulated mortality. Any early termination of an exposure will be made in agreement with the oil companies.

17. Analytical methods and monitoring of exposures (descriptions or reference).

During this exposure experiment, the following water parameters will be measured:

PAHs in water	Responsible lab: IRIS & Akvamiljø. To make sure that the concentrations of PAH are above the detection limit in the exposure aquaria we will do a pre-test before adding larvae to the aquaria. We will analyse one water sample from each concentration before we start the experiment. One pooled sample will be taken weekly from each treatment (control, 0.010 mg/L and 0.015 mg/L). The samples will be extracted using the solid phase extraction (SPE) method. In addition liquid-liquid extraction will be done on once for comparison with the SPE extraction. Although each larva will be exposed for 5 weeks, the total length of the experiment will be 6 or 7 weeks. Samples from the larvae aquaria will be taken during the whole period, - after: 1, 2, 3, 4, 5, 6 and maybe after 7 weeks, depending on the total length of the experiment.
Particle size	The particle size distribution for oil droplets in the oil dispersion will be measured in 3 replicate samples from the tube that delivers 5 mg/L oil dispersion into each mixing flask. This will be done three times during the experiment (Week 1, 3 and 5). The Coulter counter will be used to measure the size of the oil droplets.
THC	We will analyze THC by GC-FID in one sample from the CFS (in the 5 mg/L dispersion) 5 times during the experiment.

18. Analytical methods on tissue (organic matter)

The experiment will supply tissue for the following analyses:

Wet weight of pooled samples of larvae will be recorded at the end of the experiment (day 35), as in the 2007 experiment.

19. How registrations should be recorded (ex. ref. to scheme).	
Temperature	Logged daily (at one place in the water stream) and saved as Excel files. Registrations of biological data together with environmental data will be delivered to the oil companies in Excel sheet format.
20. Statistical methods	
	Standard methods/JMP: the same methods used in the 2007 shrimp larvae report.
21. Attachments (project description, exposure setup)	
Attachment 1	Test of chemical detection of nominal conc. 0.010 mg/L oil
Attachment 2	Overview of results from chemical analysis in a selection of previous BioSea II experiment.
Attachment 3	Report from chemical analysis of the new batch of Goliat oil

22. References (refer to standard methods and SOPs)

Rearing of shrimp larvae and Artemia larvae, determination of stage:
Larsen, B.K. (2004). Biological Effects of long term exposure to a North Sea and a Barents Sea oil: Early life stage tests and biomarker responses of Crustacea. Final Version. Report AM-2004/16.
Ingrid C. Taban, Renée K. Bechmann and Lars Hellgren (2007). Biosea II JIP:
Effects of Goliat oil on shrimp larvae. Internal report AM 2007 / 015.
Solid Phase Extraction of PAH in water SOP: IRIS/2.2-607

23. Signatures

Research plan are prepared according to IRIS and Akvamiljø quality procedures	Research plan are approved by	The experiment are approved by
Dato	Dato	Dato
IRIS & Akvamiljø	IRIS & Akvamiljø	Laurence Pinturier / Total Laura Bracco / Eni
Quality assurer (QA)	Experimental leader	Customer

Attachment 1

Test of chemical detection of nominal conc. 0.010 mg/L oil.

In the header tank, the concentration is normally regulated by mixing oil into the sea water supply to approximately 5 mg/L. The header tank water is distributed to the different test tanks and mixed with sea water. The flows of oily water to each tank are regulated with peristaltic pumps, while the inflows of sea water are fairly equal in each tank.

Based on the dilutions of the concentration in the header tank, the nominal concentrations can be calculated. It is necessary to measure the actual concentration because it is usually lower than the nominal due to different loss factors (sorption to surfaces and particles is assumed to play a significant role). The measurement can be done by different methods, but the one with best detection is to use PAH-components as tracers.

In a test based on the above set-up and with a nominal concentration of 0.010 mg/L oil in the test tank, the concentration of C2-naphthalene using liquid-liquid extraction was measured three times. The results were 0.021, 0.035 and 0.023 µg/l. The first measurement was at the limit of quantification. Parallel measurements using solid phase extraction, were done the first two times, and the results were 0.019 and 0.039 µg/l. Parallel control tank measurements were done at all three times, and C2-naphthalene were below the quantification limit in all samples.

This shows that chemical detection can be used down to a nominal concentration level of 0.010 mg/L oil, and that both solid phase (SPE) and liquid-liquid extraction can be used for this level (The actual concentration was around 40% of this; ~0.004 mg/L). We have previously found that liquid-liquid extraction is preferable for header tank water, and solid phase is assumed most accurate at low concentrations. The data above show that liquid-liquid extraction can also work well at low concentrations.

We therefore regard the liquid-liquid extraction method as the preferred overall extraction method when both header tank water and a range of test concentrations down to near the quantification limit should be analysed. But in the shrimp study the most important will be to be able to measure and distinguish the two test concentrations which are both near the quantification limit. If this can be achieved, measurement of header tank water not important. Both extraction methods should work, but the SPE method can be regarded as a little more secure. Since we are at the detection limit, we recommend to use both methods in parallel at one sampling.

The detection down to 0.010 mg/L oil by this procedure will also depend on the oil, and other than the C2-naphthalenes may also be used. In the test, C2-naphthalene constituted approximately 0.75 % of the oil by weight. It is reasonable to assume that a similar chemical detection will be possible for the oil to be used in the shrimp study if one of the measurable naphthalene forms is present in the oil at a comparable level.

Attachment 2

Overview of results from chemical analysis in a selection of previous BioSea II experiment.

Wolffish larvae experiment:

Mean concentration (± st. dev) of PAHs (µg/L) in water samples from the exposure tanks. The samples were taken bi-weekly (n = 4). All 4 – 6 ring PAHs were below the quantification limit in the exposure tanks. The SPE extraction method was used for the PAHs (for C1 and C2 phenanthrene no st. devs are given because they were only detected in one of the 4 analysed samples).

µg/L	Control	PW 1:1500	PW 1:500
Naphthalene	0.015 ± 0.002	0.032 ± 0.003	0.090 ± 0.012
C1-Naphthalene	0.005 ± 0.0002	0.018 ± 0.002	0.063 ± 0.008
C2-Naphthalene	0.009 ± 0.0004	0.023 ± 0.006	0.065 ± 0.008
C3-Naphthalene	-	0.013 ± 0.003	0.032 ± 0.003
sum 2 ring PAH	0.029	0.085	0.249
Fluorene	-	-	0.003 ± 0.002
Phenanthrene	-	0.002 ± 0.0003	0.005 ± 0.0001
C1-Phenanthrene	-	0.004	0.007
C2-Phenanthrene	-	0.004	0.007
sum 3-ring PAH	-	0.010	0.023
Sum PAHs	**0.03**	**0.09**	**0.27**

Shrimp larvae experiment 2007:

Mean concentration (± st. dev) of PAHs (µg/L) in water samples from the exposure tanks. The samples were taken bi-weekly (n = 4). All 4 – 6 ring PAHs were below the quantification limit in the exposure tanks. The SPE extraction method was used for the PAHs.

µg/L	Control	0.015 mg/L Goliat oil	0.06 mg/L Goliat oil
Naphthalene	0.013 ± 0.0007	0.020 ± 0.003	0.091 ± 0.02
C1-Naphthalene	0.006 ± 0.0025	0.042 ± 0.007	0.285 ± 0.05
C2-Naphthalene	-	0.095 ± 0.001	0.475 ± 0.07
C3-Naphthalene	-	0.066 ± 0.001	0.354 ± 0.04
sum 2 ring PAH	0.019	0.223	1.205
Fluorene	-	0.002 ± 0.001	0.003 ± 0.006
Phenanthrene	-	0.003 ± 0.0001	0.005 ± 0.002
C1-Phenanthrene	-	0.006 ± 0.0005	0.007 ± 0.004
C2-Phenanthrene	-	0.009	0.007 ± 0.005
sum 3-ring PAH	-	0.020	0.085
Dibenzothiophene	-	0.002	0.007 ± 0.0008
C1-Dibenzothiophene	-	0.005 ± 0.0003	0.023 ± 0.003
C2-Dibenzothiophene	-	0.007	0.030 ± 0.004
Sum DBTs	-	0.014	0.061
Sum PAHs	**0.02**	**0.26**	**1.35**

Chlamys experiment 2007:

GROUP	Low conc	Low conc	Low conc	Low conc	Low conc	Low conc	
TIME	T 2	T 4	T 6	T 8	T 10	T 12	
Compound	ug/ l	ug/ L	ug/ L	ug/ L	ug/ L	ug/ L	
Naphthalene	0.019	0.020	0.018	0.017	0.018	0.015	
C1-Naphthalene	0.026	0.017	0.021	0.014	0.026	0.012	
C2-Naphthalene	0.060	0.030	0.045	0.037	0.051	0.026	
C3-Naphthalene	0.039	0.022	0.033	0.027	0.034	0.020	mean sum PAH
sum	0.143	0.089	0.118	0.094	0.129	0.072	0.108

Attachment 3

Report from chemical analysis of the new batch of Goliat oil

Table 1. PAH analysis of raw oil samples by GC/MS. Concentration of PAH in the Goliat oil used in BioSea I (analysed in 2003) and in phase 1 of BioSea II (analysed in 2006) compared to the new batch of Goliat oil received in week 6 (2008) from Eni.

Analysed, year	2003	2006	2008
Sample code	AM 03 285	AM 06 178	AM 08 058
Sample name	**Raw oil**	**Raw oil**	**New batch**
Sampling date	01.12.03		05.02.08
Compound	mg/kg	mg/ kg	mg/kg
Naphthalene	1271	1558	985
C1-Naphthalene	4569	4815	2694
C2-Naphthalene	6490	6655	4196
C3-Naphthalene	4680	4509	2834
Acenaphthylene	9	mi *< (16)	mi *<
Acenaphthene	9	17	mi *<
Fluorene	9	140	73
Phenanthrene	222	230	170
Anthracene	*<	*<	*<
C1-Phen/Anthr	368	440	275
C2-Phen/Anthr	309	403	307
Dibenzothiophene	95	117	25
C1-Dibenzothiophene	268	308	69
C2-Dibenzothiophene	265	333	70
Fluoranthene	3	5.9	6
Pyrene	8	8.7	5
Benzo(a)anthracene	2	3.7	3
Chrysene/Triphenylene	8	11	9
C1-Chrysene	15	24	21
C2-Chrysene	18	27	24
Benzo(b,j)fluoranthene	*<	3.9	4
Benzo(k)fluoranthene	*<	*<	*<
Benzo(b,j,k)fluoranthene	*<	4	5
Benzo(a)pyrene	*<	5	2
Indeno(1,2,3-cd)pyrene	*<	*<	*<
Benzo(g,h,i)perylene	*<	*<	*<
Dibenzo(a,h)anthracene	*<	*<	*<

LOQ 2-4 mg/kg

06.02.2008

mi: matrix interference

***< can not be quantified**

The new oil has a lower concentration of PAH than the oil used in the previous experiment (analysed in 2006).

The 2006 oil had:

- Approximately twice as much naphthalenes, fluorene, C1-phenanthrene and pyrene per gram oil as the 2008 oil.
- 4-5 times as much DBTs per gram oil as the 2008 oil.

Chromatogram of old (blue line) and new (black line) raw oil.

The new sample contains much more of the lighter oil fractions, e.g n-alkanes C9 to C14 as the chromatogram shows. The nC17/Pristane ratio is also different. The total PAH content is about 30% lower in the new oil sample.

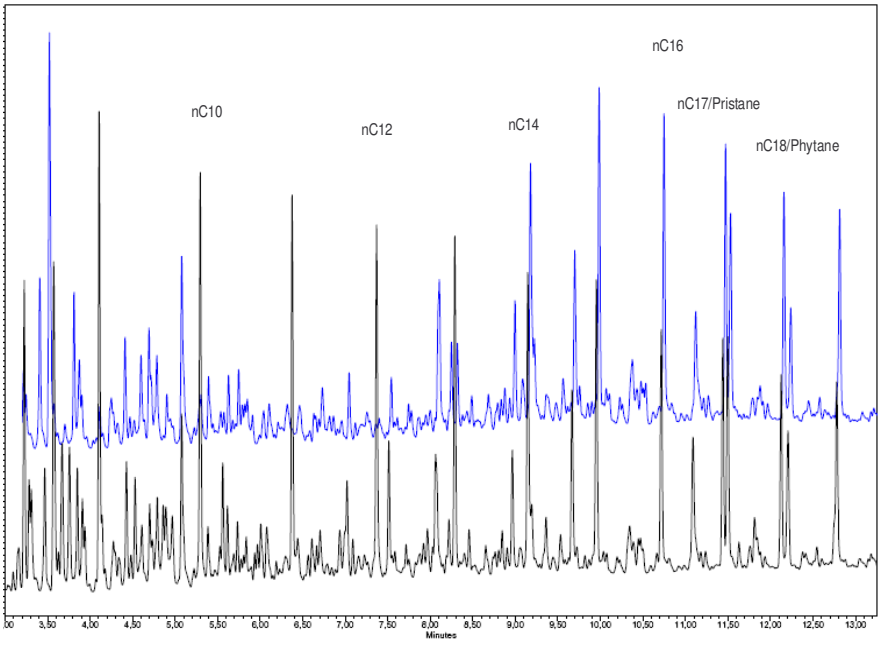

Figure 1. The new oil contains much more of the lighter oil fractions, e.g. n-alkanes C9-C14 as the chromatogram shows. The nC17/Pristane ratio is also different. The total PAH content is about 30 % lower in the new oil sample.

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